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Chemical warfare: rearmament or disarmament?

THIS past week a party of diplomats from a wide range of countries has been flying around Britain looking at the demolition of a chemical weapons factory in Cornwall and inspecting the Birmingham premises of a manufacturer who handles phosphorus compounds. This somewhat unusual exercise is part of an Anglo-German effort to put the problems of chemical disarmament in clearer context. But despite occasional flurries of activity, indeed despite draft treaties tabled with the Conference of the Committee on Disarmament, there is as yet nothing like unanimity amongst nations on what sort of agreement would be acceptable, and there are substantial pressures, most notably in the United States, for chemical rearmament.

Biological weapons were effectively banned by a 1972 convention in which all major states agreed 'never in any circumstances to develop, produce, stockpile or otherwise acquire or retain microbial or other biological agents, or toxins . . .'. Additionally the Geneva Protocol of 1925, also widely adhered to now, prohibits the use in war of 'asphyxiating, poisonous or other gases' although this protocol does not close the door on research and development, production or stockpiling of chemical weapons against possible first-use by another nation. It is this lack of prohibition on 'defensive' chemical weaponry which has allowed the development of mustard gas, hydrogen cyanide and nerve agents, particularly sarin, soman and VX, together with the means of delivery. It is known that the Soviet Union, France and the United States maintain stocks of chemical weapons; whether any other nation, particularly in the Middle East, possesses them is unclear. However, material captured during the 1973 Middle-East War showed that the Soviet Union was prepared to supply allies with the wherewithal to fight in a toxic environment.

Since then the United States has been taking serious stock of its ability to cope with a chemical warfare attack and, if necessary, to retaliate in kind. Binary weapons, comprised of two harmless constituents which mix in the missile only after launch, are being discussed seriously (*Nature*, June 15, 1978, page 481). And this past year two important documents have been published: a plea for the US government to give chemical warfare much more attention ('The Neglected Threat of Chemical Warfare, by A. M. Hoeber and J. D. Douglass, in *International Security*, Vol. 3, No. 1) and the proceedings of a conference which looked at the wide spectrum of possibilities for responding to Soviet activity, in particular by arms control measures (*Chemical Weapons and Chemical Arms Control*, Matthew Meselson, ed., Carnegie Endowment, 11 Dupont Circle, Washington, DC).

The most likely venue for any chemical attack would be central Europe, and the most likely target would be small pockets of soldiers, or military installations. A chemical strike against an airfield might be particularly effective. There are clear similarities between chemical and tactical nuclear weapons, but, of course, an obvious distinction is

that once the nuclear road had been taken in a war the possibilities of escalation would be almost infinite.

This poses a dilemma: if the Soviet Union were to lead with a chemical attack, would NATO respond in kind or would it use tactical nuclear weapons? There is a fair amount of disagreement about the answer. The United States maintains large stockpiles (almost all back home at present) and is busy equipping its soldiers with new protective clothing; its army would undoubtedly like to have the capability of responding in kind. On the other hand West Germany does not store chemical weapons and does not train personnel to use them. Britain too does not maintain retaliatory capability, relying on nuclear deterrence, but also stresses the value of anti-chemical defences (having been successful in selling protective clothing to her allies).

There is even disagreement about the size of the Soviet threat. Hoeber and Douglass speak of Soviet superiority over the United States being of 'two or three orders of magnitude', making chemical warfare, even uncoupled from nuclear attack, 'a major contingency for which the Soviet Union plans'. On the other James Leonard, an arms controller, speaks of a *de facto* chemical disarmament in Europe and says he has never been satisfied that there was a significant Soviet capability to wage a chemical war, whilst Julian Perry Robinson, of Sussex, asserts that the threat of Soviet chemical weapons 'can at most be marginal'. Faced with a diversity of assessments (based, it must be said, on desperately little hard data) and faced with an alleged aversion, even by military men, to contemplating the use of such unchivalrous means of warfare, is it possible to neutralise the chemical weapons threat, not by pressing on with bigger and better stockpiles, or by improving protective clothing, but by arms control measures?

The military man will say: yes, but first have a strong retaliation capability as a bargaining chip; on the other hand the arms controller might even propose unilateral measures of disarmament to build confidence. Between nations, however, the sticking point is the difficulty in achieving any satisfactory degree of verification. The Soviet Union's favoured brand of monitoring is self-monitoring. Western proposals are much tougher on international safeguards, such as on-site inspection. A resolution of these differences does not seem imminent.

In the meantime, what does Britain do? At present her initiatives seem to be in promoting chemical defence and arms control. But this supposes that retaliation by nuclear weapons will be effective and acceptable. Could Britain start work on chemical offensive weapons again? Not under a Labour government, but might a Conservative government sanction modest activities? We simply don't know. Public discussion of chemical warfare is just about non-existent in Britain; presumably in the restricted circles of ministers, generals and top civil servants some people are thinking hard about options. It is time the debate was widened. □



Einstein: disagreement delays publication of collected works

David Dickson reports on the circumstances that have delayed the publication of Albert Einstein's collected writings, which fill 28 file drawers at Princeton University

PLANS to produce a multi-volume edition of Albert Einstein's published and unpublished writings are virtually at a standstill following the failure of the trustees of Einstein's estate, the Princeton University Press, and the project's editor to agree on mutually acceptable terms under which the editorial work can proceed.

Already a project funded by the National Science Foundation, whose initial purpose was to prepare the papers for publication, has had to be revised to the more modest goal of indexing and preparing duplicate copies of the papers. These are kept at the Institute for Advanced Studies in Princeton, where Einstein worked after coming to the USA.

There are no plans at present for the NSF to provide further support for the project after the current contract of the editor of the collected works, Professor John Stachel, expires on 14 July. Nor has the university press announced how it plans to pursue the project after this date.

The trustees of Einstein's estate, who have never refused access to the papers by "reputable scholars", have taken great care to ensure that the editorial work is carried out in a way that they feel would have met his approval. (Dr Otto Nathan, one of the two trustees, has expressed strong disapproval of the National Academy of Science's plan to erect a statue of Einstein in Washington, since he claims this is explicitly against the physicist's wishes).

However a number of physicists and historians of science, many of whom are reluctant to be quoted for fear of exacerbating the current situation, are concerned that the trustees have interpreted their responsibilities so rigidly as to restrict a full appreciation of Einstein's contribution to twentieth century science and thought.

Dr Paul Foreman, for example, organiser of a current exhibition on Einstein at the Smithsonian Museum in Washington, has expressed his regret that the trustees have refused, as a matter he claims of "unbending policy", to loan the exhibition letters and photographs from the estate.

Albert Einstein was a prolific writer on both scientific and non-scientific issues. During his lifetime he published 274 scientific papers and 332 papers of

a general content; these, and a large quantity of unpublished material, form the archives which currently fill 28 file drawers at Princeton University.

His correspondents range from scientists such as Erwin Schrodinger, Max Planck, Wolfgang Pauli and Marie Curie, to contemporaries in other fields, such as Sigmund Freud, Bertrand Russell, Franklin Roosevelt and George Bernard Shaw. And some of the letters, particularly to physicists, suggest further avenues of research which Einstein himself was unable to follow.

On his death in 1955, the literary rights to his papers passed to a trust, of which the two trustees are Helen Dukas, his secretary from 1928 to 1955, and Dr Otto Nathan, an economist and close friend who, like Einstein, came to the US as a refugee from Europe. (Under the terms of the will, the beneficiaries of the trust are Miss Dugas and Margot Einstein, his stepdaughter; eventually the estate will pass to the Hebrew University in Jerusalem).

Negotiations over the publication of the collected works began within weeks of Einstein's death. In 1971, the trustees reached an agreement with the Princeton University Press, which had expressed great interest in the project from the very start. And in 1976 the trustees and the press appointed Professor John Stachel, a physicist on leave from Boston University who has recently co-edited selected papers of Leon Rosenfeld, to be the editor.

In the same year, the university press submitted a grant proposal to the National Science Foundation, requesting \$787,300 over a five-year period to support editorial preparation for the multivolume project. This, they suggested, would be a "fitting monument" to Einstein, and would include all his published and unpublished writings in the original languages, with English translations of some of the more important works.

The foundation agreed to provide initial support to allow Professor Stachel to plan the editorial work, and to establish the general principles of editing the whole project. The foundation awarded \$80,000 towards this in 1977 and 1978. But difficulties soon arose in agreeing on a work plan that would be acceptable both to the

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Lotte Jacob

Prolific writer: 606 papers published

trustees and to Princeton University press.

Dr Nathan insisted, for example, that only the editor be allowed to handle the original documents, thus making it necessary for Dr Stachel to do much of the work that would otherwise have been done by research assistants. And other areas of disagreement arose over how the editorial work on the documents should proceed.

It soon became clear to the participants that it would be impossible to proceed with the preliminary editorial work plan that had been developed under the NSF grant, or to complete negotiations with the foundation on the initial five-year grant proposal (even though the plan had been endorsed by an editorial advisory board which met to discuss the situation early in 1978).

At the prompting of NSF's review panel, the university press and Professor Stachel approached the foundation again to support the completion of work on a duplicate archive and an index, to make sure that "when the time comes, the editing of the Einstein papers can proceed without the past year-and-a-half being wasted".

The trustees and the press wrote in a joint statement that a duplicate archive was "absolutely necessary for the continuation of the editorial work if we are able to proceed after 14 July 1979," but added that "if the work of editing *The Writings of Albert Einstein* should cease, one copy of the entire duplicate archive will be kept intact and unmarked so that a new editor, when appointed, could use it with confidence".

Work is now almost completed on the material for the duplicate archive being carried out under an NSF grant which runs out on 30 June; so, too, is the preparation of an index to all the

papers in the archives, the total number of which may be as high as 60,000. And Professor Stachel has also been able to contact many of those who were in correspondence with Einstein, asking for their comments on copies of letters which have been sent to them.

But there the matter rests. Mr Herbert Bailey, head of the university press, admits that the project has had "problems", and does not accept that it has gone into abeyance. "We have a lot of reasons to think that the project, on which we are as keen as we have always been, will go forward" he said last week; however he declined to reveal how the project will proceed after the NSF grant money, and Professor Stachel's contract, run out in the summer.

Dr Nathan is equally adamant that claims that the trustees have placed unworkable restrictions on how the editing work should proceed are "nonsense", and that the trustees "are being maligned" if such charges are being made.

He says that since a few days after Einstein's death the estate has been attempting to get a complete edition of the physicist's writings published, but that he has been "blocked again and again", and that he feels that "at the moment we do not know where we are going".

Historians of science are hoping that the stalemate can be resolved by some means (there are rumours of possible litigation, but these cannot be confirmed). No one disputes that the

collected works, when they are eventually published, will be a major contribution to twentieth century scholarship, helping to provide a far greater understanding of Einstein's role in the development of both modern science and the modern world; but few do not express frustration at the current impasse.

"When completed it will be—or should be—what historians and philosophers of science, and others, have been waiting for since Einstein died", Dr Jeremy Bernstein, professor of physics at Stevens Institute of Technology, and author of a biography of Einstein, wrote recently. "Not all this material is of great interest . . . but what matters is that it should be available." □

FDA scientists dispute ethics of testing laetrile

A SCIENTIST with the Food and Drug Administration has filed a "citizen's petition" against his agency in an attempt to prevent it from giving approval to clinical trials which the National Cancer Institute is planning to carry out on the controversial anti-cancer drug laetrile.

Dr Robert Young, a clinical oncologist with the FDA, echoing concerns which have already been expressed and debated within the NCI, claims that such trials would be in violation of accepted ethical codes since no firm scientific evidence yet exists that the drug has any beneficial effects.

Both the FDA and the US medical establishment maintain stiff opposition to the use of the drug, which is derived from apricot kernels and is currently taken by thousands of cancer patients in the nation. The agency has issued notices warning that laetrile is "worthless" and that its cyanide content in particular is potentially dangerous.

However despite this uncompromising stand, FDA commissioner Dr Donald Kennedy, previously professor of human biology at Stanford University, has come under pressure from those who argue that although previous studies do not provide any conclusive evidence that laetrile has a beneficial effect, neither is there sufficient evidence to rule out such an effect.

The clinical trials have been proposed by the NCI following a retrospective study of laetrile patients which the institute, having frequently rejected the idea in the past, agreed to carry out last year.

Over 90 cases claiming evidence of a beneficial effect of laetrile treatment were submitted in response to a nationwide appeal, and a detailed study of 67

of these by a panel of oncologists revealed six patients who had received laetrile as their primary treatment, and were judged to have had a clinical response.

Announcing the results of the study last autumn, the NCI said that these would not normally be sufficient to suggest that a drug merit clinical tests over other candidate drugs. However it added that "because of widespread public use and interest in laetrile, the NCI will proceed with plans to evaluate the drug".

Subsequently the institute filed an "investigational new drug application" (IND) with the FDA in December. And although the agency has taken considerably longer than normal to approve the application—there have been detailed discussions with the NCI both about the protocol for the experiment, as well as on technical details about the quality, stability and purity of the substance to be tested—this is expected to be granted within a few weeks.

Dr Young's objection to granting approval is that "there is not adequate scientific data that would justify the

test of the drug in human beings."

In an interview with *Nature* last week he cited in particular the lack of evidence, accepted by the NCI, of the drug's efficacy in animal tests; and even the retrospective tests, he says, provides "no scientific data from which you can responsibly conclude that it was the taking of the drug that mitigated the disease".

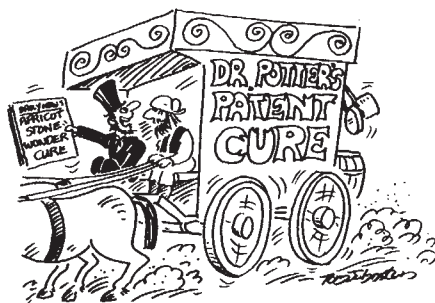
In his petition, which Dr Young says reflects the feelings of a number of laboratory staff in the agency, he requests that laetrile should not be exempted from the provision of the Food and Drug Act, which require reports "adequate to justify the proposed clinical testing". And he criticises the agency for using a "sociopolitical argument", rather than a solid scientific case, to justify the trials.

If the FDA approves the cancer institute's IND, a six-month study will be carried out involving between 150 and 300 patients with different types of cancer; participation will be restricted to patients in whom all known therapies have been attempted.

Meanwhile the Supreme Court has agreed to rule on whether the FDA has the right to bar the use of laetrile as an anti-cancer drug, following the decision of a lower court that the need to show a drug is both "safe" and "effective" before being approved for sale or distribution does not apply to drugs used by terminally ill cancer patients.

In appealing the decision to the Supreme Court, the FDA has claimed that this ruling seriously limits its power to protect the public from unsafe and ineffective drugs, adding that it would be "virtually impossible to restrict the use of laetrile to the terminally ill".

David Dickson



"To the nearest apricot farm!"

Space shuttle: costs and technical delays raise political doubts

AFTER almost 10 years of preparation, technical delays and uncertainties are turning the final development stages of the National Aeronautics and Space Administration's Space Shuttle programme into something of a political cliffhanger.

A succession of last minute technical problems, although not unusual in a project as complex as developing a re-usable launch vehicle able to place and subsequently retrieve objects from an earth orbit, are receiving wide press attention and close public scrutiny. And Congress has already told NASA that, in a year of tight budgets, the agency cannot be guaranteed of receiving the extra \$185 million it has requested for covering the extra work involved. Current estimates of the cost are 8-9% higher than originally estimated in 1971. If granted, the additional money would take the total science budget for the financial year 1979 above a previously agreed ceiling.

If NASA does get the extra money, it is confident that Space Shuttle can be in orbit by the end of the year. Even though it publicly admits that the chances of meeting the planned 9 November launch date are "relatively low", it has criticised the National Academy of Sciences which suggested a launch date of April 1980, as being "unduly pessimistic".

But if NASA does not get the money—and the request is being closely scrutinised by the Senate budget committee, in particular since money for space research comes under the heading of discretionary funding—then the agency could be in deep trouble.

Under such circumstances, according to associate administrator John F. Yardley, the first manned orbital flight would be delayed an additional six to nine months. The delivery of production orbiters would be delayed up to one year, and this could seriously disrupt planned launch schedules.

Among the projects affected could be the European Space Agency's Spacelab, whose first launch is currently scheduled for August 1981. Initially planned for launching at the end of this year, cost increases for Spacelab—some made necessary by changes in the design of the Space Shuttle—have already exceeded the limit above which participating nations are entitled, if they wish, to withdraw.

Since being given the go-ahead by President Nixon in 1972, the Space Shuttle programme has had to run a continuous gauntlet of congressional criticism. Among past critics is Vice-



President Walter Mondale, today silent on the issue, but in 1972 claiming that the Shuttle was many times worse than supersonic transport "in the magnitude of its cost, in the folly of its concept, and thus in the damage to the country."

Such opposition has kept the research and development programme to a skeletal minimum, a far cry from the lavish spending on the Apollo moon programmes. Mr Yardley complains that requirements carried out in 1974, 1975 and 1976 have taken their toll, and that "further reductions in programme content are not considered possible".

Funding pressure has forced hard managerial decisions on NASA, and some of these were commented on by an *ad hoc* review group set up last year at the request of a senate committee by the National Academy of Sciences under the chairmanship of Dr Eugene E. Covert, professor of aeronautics and astronautics at Massachusetts Institute of Technology.

In an initial report last March, the committee pointed out that unlike previous programmes, the development strategy for the Shuttle programme as devised by NASA was "success oriented", and based on the assumption that each piece of development hardware and its subsequent verification test would succeed at the first attempt.

"It may be that this strategy has already saved time and money However, if or when malfunctions occur during the testing of the operational engine, new hardware may need to be designed, constructed and retrofitted, causing delays in the programme", the report said.

Such indeed has turned out to be the case. There have been problems, for example, both with the turbine blades and with the heat exchanger in the Shuttle engine being developed by Rocketdyne—the latter being a component which the review group singled out for concern. And the temporary tiles which broke loose during a flight last week and damaged some of the fitting had been made necessary by un-

expected delays in fitting the permanent tiles—of which there are 5,000, each requiring 650 man-hours of work.

There have also been problems with weight. Design changes in the launcher vehicle have caused problems for, for example, the Galileo orbiter probe to Jupiter, planned for launch in 1982, but on present specifications now requiring 109% of the engine's theoretical thrust.

The big question now is how smoothly the rest of the pre-launch development programme goes. NASA admits that its present schedule is a "success oriented schedule with a minimum of contingency time", but adds that "it has been our experience in the past that such success schedules result in minimum cost and earliest actual dates",—in other words that even if unrealistic, planning for sooner rather than later helps to keep the pressure up.

The Covert committee is less optimistic. "We think it highly likely that there will be more problems before the first orbital flight", one member of the committee said last week. This sanguine perspective led the committee last month to recommend next spring as a more realistic date. NASA says that this is based merely on "intuitive feeling".

Meanwhile NASA officials are busy trying to persuade Congress to give them the extra money needed to let the shuttle programme proceed to its conclusion as planned. The supplemental request is being examined for Senator Muskie by the General Accounting Office of Congress. The 1980 request for NASA contains no new space science starts largely because of the pressure on Shuttle funding.

There is already talk of NASA possibly needing a supplemental to its 1980 budget, even though it may mean the agency coming back to Congress cap in hand. Ten years ago, the money would have been provided eagerly—today no-one is taking bets on the outcome.

David Dickson

West Germany: eyeball to eyeball over nuclear fuel reprocessing

NEXT week, critics and supporters of nuclear power will be meeting in Hanover, West Germany to analyse proposals to build a nuclear fuel reprocessing and waste disposal facility at a site 5 km from the East German border near the village of Gorleben in Lower Saxony. The forum for the analysis is to be a week of public hearings organised by the state government of Lower Saxony.

Twenty-five expert nuclear critics, 20 of them from outside West Germany, will face 25 nuclear supporters in a 'round table' discussion: an audience of 200 will listen in. The ostensible aim of the hearings is to inform the public and to help Ernst Albrecht, premier of the Lower Saxony government, decide whether or not to grant a licence for construction of the facility.

Already there are fears that the hearings will get off to a bad start. Some of the critics are suspicious of the state government's motivation for holding them: they think that pressure from the federal government means that construction of the plant is inevitable. They are also critical of the delay in deciding on a format for the hearings and of the lack of public information on them. Three weeks before them, the names of the 25 pro-nuclear experts had not been released, although the anti-nuclear names had been known for a long time.

Criticism has also been levelled at the way in which the 200 places in the audience have been allocated. Apparently about 100 places are to be taken by state and government authorities, 20 by representatives of the nuclear industry, 30 by the press, only three by members of local environmentalist groups and one by a representative of the district authority. The remainder are for members of parliament of Lower Saxony. With no spare places for interested members of the public and only 30 press places, it is argued, the hearings are not truly public.

The proposal under discussion is for the construction of a plant capable of reprocessing all of West Germany's spent nuclear fuel. It would include spent fuel storage ponds and plants for fabricating mixed oxide fuel, uranium finishing, vitrifying high-level radioactive waste as well as reprocessing 1,400 tonnes of spent fuel per year. Final disposal of all radioactive waste would also be on the site in salt domes a few hundred metres below the surface. The total cost is estimated at DM12,000 million.

The nuclear industry, amongst others, expects the decision reached by the Lower Saxony government over the licensing of the plant to be crucial to the future of nuclear power in West Germany. In the summer of 1977, the federal government announced that no new reactors could be licensed until the problem of disposing of spent nuclear fuel—*Entsorgung*—had been solved. Since then, reprocessing has generally been assumed as the only way of satisfying that condition. Unless an alternative can be found (and the nuclear critics hope to show next week that there is one) a veto of the reprocessing plant is expected to mean an end to the expansion of nuclear power in West Germany.

Plans for the proposed facility, or *Entsorgungszentrum*, were first submitted to the Lower Saxony government in March 1977, by DWK, a company set up by the West German utilities specifically to build it. At the same time, plans for the underground waste storage facility were submitted by the federal Physical-Technical Institute. A 3,000-page safety report, which accompanied the two applications, was examined by the Commission for Reactor Safety and the Commission for Radiological Protection, two advisory bodies to the federal Ministry for the Interior. In October 1977, both bodies published their comments on the safety report saying that they found it reliable and acceptable. The federal government, believing that the disposal of spent nuclear fuel was under control, began to talk about expanding the nuclear programme.

Meanwhile, local people in Gorleben started to protest about the proposal. They felt that the two commissions had been biased in their assessments of the safety report. In addition, DWK had started to try to purchase land from local landowners by offering them a very high price if they sold by a certain date. If they missed the dealing, DWK threatened to start expropriations procedures to procure the land for a much lower price.

To date only 40% of the land has been sold. Of the remaining 60%, the vast majority is owned by one man—Count Bernstorff, a remnant of the German aristocracy and prominent figure in the local landowner association (which has been very active in organising the protest).

At the beginning of 1978, the landowners association began to put pressure on the Lower Saxony govern-



ment to set up a committee of independent nuclear experts to review the proposals. After approaching one or two foreign experts to gauge support for the idea, the association drafted a list of experts it wanted to sit on a review committee. After elections in June of last year, when local environmentalist groups took 18% of the votes in Gorleben and 3.9% in the surrounding area, the state government agreed to invite the experts to spend 30 days producing their own report on the proposals and safety report.

In September 1978, 20 expert nuclear critics from France, Norway, Sweden, the UK and the US met in Hanover for the first time. By the beginning of March they had prepared a 2,200-page report and had submitted it to the Lower Saxony Ministry of Social Affairs. The report consists of eight chapters each prepared by different experts. The first chapter is a general introduction, the second discusses routine emission of radiation and radiological protection, the third accidents, the fourth safeguarding of fissile materials, the fifth the technical problems of reprocessing, the sixth waste management, the seventh final disposal and the eighth decommissioning. Among those taking part in the review were Walt Patterson and Amory Lovins of Friends of the Earth, Frank Barnaby of the Stockholm International Peace Research Institute, Paul Sieghart of the International Commission of Jurists and Professor O. Lindstrom of the Royal Institute of Technology, Stockholm.

Although the members of the review panel started work last September, they did not receive their contracts until the end of December. Several of them claim that during discussion of the terms of the contract they had requested that the report be published before the public hearings. However, the contracts said that the report had to be withheld from publication until 10 July.

A significant achievement of this action has been to increase dissatisfaction with the way the review and hearings are being run. The state government is committed to take some decision on the future of the reprocessing plant in May or June, before

the July publication date. Some feel this defeats the object of the review as a public information exercise.

More generally, there are feelings that the government would not seriously contemplate calling a halt to the plant whatever the outcome of the hearings. The formal licensing procedure was begun in 1977, and although it has now almost ground to a halt,

it is thought that the Lower Saxony government would be unlikely to stop it abruptly. Even if licensing were now to proceed as fast as possible, it would be 1981 before it were completed. What seems most likely, therefore, is a 'vague' decision.

Whilst the critics and nuclear industry are discussing the Gorleben proposals next week, the people of Gorle-

ben, who do not have an invitation to the hearings, will be taking part in a demonstration march from Gorleben to Hanover. The Lower Saxony government is expecting the affair to be violent for it is reported that 100 prisoners have been transferred from local gaols to make way for the recalcitrant villagers.

Judy Redfearn

US panel approves research on *in vitro* fertilisation

THE US is likely to end a three-year moratorium on research on the *in vitro* fertilisation of human ova following the conclusions of a federal advisory board that there are no ethical objections to such research being carried out on federal funds.

The ethics advisory board was established last year by Mr Joseph A. Califano Jr, Secretary of Health Education and Welfare, to advise him on whether work along the lines of the British research workers Mr Patrick Steptoe and Dr Robert Edwards should be permitted to proceed in the US. On reaching its conclusion, however, the board declined to say whether it felt that such research should in fact receive federal support.

Having heard evidence from scientists, religious leaders, anti-abortion groups, infertile couples and others, the board recommended that the work should be permitted provided that a number of requirements were met. These include:

- that research be aimed primarily at establishing the safety and efficacy of procedures for *in vitro* fertilisation;

- that no embryo be sustained in laboratory conditions for more than 14 days;

- that both the participants in the research—which would include clinical trials—and the public be informed if there is any evidence that such research is having ill-effects.

Although these provisions, and the decision to recommend that the moratorium be lifted, were agreed unanimously by the 13-member board of lawyers, scientists and others, there was less agreement on the priority which *in vitro* fertilisation should be given as a research topic.

Several members suggested that it should come low on the national priority list, and one suggested that the government should not fund any such research. Others disagreed, claiming that the research might soon become a "high priority" if there is wide demand from doctors to adopt the new techniques.

The board therefore agreed not to recommend whether HEW should fund such research, accepting that in addition to ethical concerns, this also

raised scientific, economic and political considerations.

Funds for 'test-tube baby' research have been held up for more than three years in anticipation of the advisory board's report, although the members of the board were only announced last summer. Congress placed a total ban on all research involving fetuses in 1974, and although this ban was lifted one year later, the position with respect to *in vitro* fertilisation has remained uncertain.

The only application at present lodged with HEW for carrying out such research is from Dr Pierre Soupart of Vanderbilt University, who has been waiting since 1975. The ethics board suggests that Dr Soupart's application now be forwarded to the National Institutes of Health for scientific review.

The board also recommended that the secretary of HEW should encourage, and even possibly provide financial support for, the development of a model law outlining the legal status of children born as a result of *in vitro* fertilisation. □

Spanish universities unhappy with new autonomy law

SPAIN'S universities have long sought autonomy from government control—and they should achieve it as one of the first actions of Spain's new government. But it may not prove to be the prize once dreamed of.

The first duty of the new government will be to debate the laws outlined in Spain's new constitution; and one of these is a law granting university autonomy: but was the law well-framed?

Government officials, political parties, and university professors are unanimous in their belief that the universities need rapid attention: they suffer from overcrowding, graduate unemployment, poor teaching, and lack of research. Furthermore the universities do not have the power to distribute their own funds, to select their staff or students, or to organise their own teaching. Every decision has to pass through the central Ministry of Education.

Even before the Constitution was written and approved, the Ministry of Education and Science was preparing a

draft of a law for autonomy, and—despite strong criticism—it was forced through the Council of Ministers and entered parliament the day before its dissolution.

The law as it has been published is a long and in certain aspects detailed text. It deals at length with the structure of university management (Senate, Rector, etc.) and with the teaching staff; but it deals only briefly with the administrative staff and research.

Two changes introduced by the new law are financial autonomy, and the separation of academic and economic authorities. The law also allows the existence of private universities, which would interest certain religious societies.

But many points in the law have received criticism from the universities—such as the system of selection of teaching staff, which remains almost untouched. For example, an aspirant to full professorship (Catedrático) must spend three years as associate professor (Adjunto), a regulation which bars many non-academic professionals

whose expertise might benefit a university.

The law also describes—in detail—the composition of the various university institutions, making it difficult to apply to any new university, while some fundamental problems—such as the role and funding of research, the selection of students, and increases in funding or staff, are left untouched.

The parliament formed after the elections held on 1 March is almost identical to the previous one, and the centrist party will most likely be charged with the task of forming the new government. It will probably back the existing law as created by the Ministry.

In the universities it is hoped that the law may produce some positive changes, but it is regretted that a general debate and a deep rethinking of the function of the universities in Spain has not taken place. If the law is approved as it is, it may be felt by the teaching community as a lost opportunity.

Pedro Puigdoménech

Bulgarian industry tackles environment

BULGARIAN industry is involved in a crash programme of anti-pollution measures, according to Mr Vordan Tsvetanov, Deputy Minister of Machine Building, who visited Britain recently.

The visitors, who included the Minister of Machine-Building, Mr Toncho Chakarov, came to discuss increased trade and cooperation in internal combustion engines, hydraulics and electro-technical equipment. Although pollution problems did not enter specifically into these discussions, Mr Tsvetanov told *Nature* that Bulgaria was making "wide use" of the anti-pollution experience of other countries and was naturally very interested in possible cooperation "especially as far as production facilities are concerned".

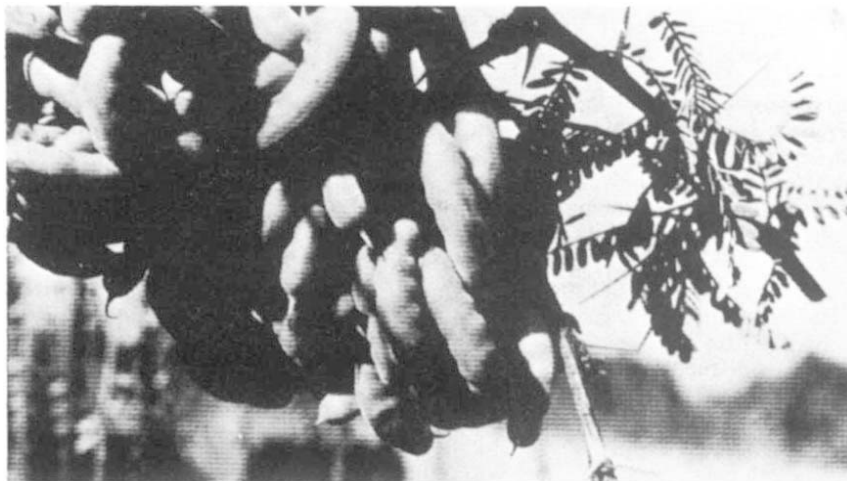
According to Mr Tsvetanov, new industrial complexes in Bulgaria pose little threat to the environment. A special government body "has a decisive say" in all matters touching on potential hazards "as early as the planning stage", deciding on the siting of the plant and the anti-pollution measures and equipment to be used. The main problem, he said, is that of existing plants which do not meet the more stringent modern requirements; Bulgarian industry is developing very rapidly and, accordingly, "everything has to be brought up to standard as soon as possible".

His own ministry, said Mr Tsvetanov, was particularly concerned with the purification of gaseous and liquid effluent. There is a "very substantial investment" in anti-pollution equipment, he added, "approximately 5%" of all capital invested in industry. He stressed, moreover, that his ministry's work is only part of an over-all plan for environmental protection.

Asked whether Bulgaria had made any major innovations in conservation and anti-pollution measures, Mr Tsvetanov said that it was "very difficult to say" if Bulgaria had achieved any major breakthrough.

This was a surprisingly modest remark in view of the numerous successes announced in the Bulgarian media. During the past month, Bulgarian radio has described new closed-cycle effluent-free production processes for copper, zinc, lead and molybdenum developed by the Bulgarian Academy of Sciences, a "superior" new coagulant for use in water purification, likewise from the academy, and an "efficient" method of recovering mercury from effluent, developed by the Sofia Institute of Chemical Engineering.

Vera Rich



Tamarugo, a high-protein leguminous plant which survives Chile's Atacama desert. Research on species such as this could bring great benefits.

"A goldmine" for scientists in third world

"THE vast majority of agricultural research has been on north temperate species in north temperate research stations. This research has been done by the countries that need it least." Yet, 85% of all plant species grow in the tropics. This is "a goldmine of new material waiting to be worked", declared Arthur Bell, Botany Professor at King's College, London.

He was speaking in London Saturday at a symposium on "What kind of science for African Development?". The meeting is intended to be the first of a series run by the UK African Studies Association.

Bell also argued that research has concentrated on cereals, because they are more important in north temperate zones, whereas legumes are more important for developing countries, which are usually tropical. Legumes fix their own nitrogen, reducing the demand for chemical fertilisers, they are rich in protein, and they grow in the most inhospitable places.

Of the 17,000 species of legumes, most grow in the tropics. But few kinds of legumes are eaten, because most are toxic to man. Some have been bred over the centuries: lima or butter beans now contain less than 3% of the cyanide of their natural Javan ancestor.

He suggested that research should concentrate on already existing minor crop plants to make them more acceptable. "One can improve a plant in its own habitat with many fewer gene changes than by bringing a plant from far away in Rothamsted", UK experimental station.

One example, based on his own research, is the *Lathyrus* of which most species are toxic. (The common sweet pea is a *Lathyrus*). In India, various *Lathyrus* species are commonly used

for food, but if eaten in large amounts cause a paralysis of the legs known as Lathyrism. Researchers found that the toxin was oxalyldiaminopropionic acid. Bell showed that it was easy to use high voltage electrophoresis to test for this acid, and that not all *Lathyrus* species contained it. In a paper in *Nature* more than 15 years ago, he suggested that India substitute non-toxic *Lathyrus* species.

He now admits that this was a naive suggestion. The three commonly eaten toxic species grow well and were rich in protein. But a variation of his suggestion was adopted. Indian agricultural scientists used high voltage electrophoresis to find that there was great variation in the toxin within the species, and are now substituting less toxic varieties. Similar work has been done in Australia to make *Leucaena* less toxic to animals.

Bell's concern about the research bias toward the developed countries was picked up by other speakers. Indeed, the conference convener Paul Richards noted that although Bell's work was of direct use of developing countries, it was done in London rather than a developing country.

Barbara Harriss of the University of East Anglia, in a paper on the Sahel submitted to the symposium, pointed out that research funded by Western agencies tended to support particular policy aims. For example, the World Bank was looking for technological packages that the first world could export to the third. And aid donors tended to support government marketing and agricultural structures. Yet as a rule neither of these help the poorest peasants. Indeed, research has ignored what the peasants actually eat—their nutrition and preferences and what factors affect their food consumption.

Joseph Hanlon

Joseph Hanlon is a development journalist specialising in third world science.

news in brief

Japanese physicist in UK fights deportation:

Dr Tetz Yoshimura is neither a political refugee nor a victim of economic or racial pressures—simply a mathematical physicist who wishes to pursue his researches unhampered by the materialistic rat-race of modern society. For this reason he left his native Japan in 1963, and, after holding a number of academic appointments in various countries came to the UK in 1971. Britain, however, is now proving a reluctant host, and Dr Yoshimura is fighting a deportation order.



After two years as a research fellow at the University of Essex and Kings College, London, Dr Yoshimura adopted a freelance life, concentrating on his research and supporting himself by freelance translation and the like. By drastically paring down expenditure on creature comforts, he claims that he can maintain himself on £12 per week, and that his average income is usually comfortably double this sum.

The Home Office, however, demand proof of a regular £100 per month before they will give him the papers necessary for permanent residence. Furthermore, since his original entry permit described him as a "student", his original application for permanent residence was rejected, apparently as a matter of routine.

Dr Yoshimura is an active researcher in axiomatic quantum field theory with papers published in 1978-79 in the *Physical Review*, *Journal of Mathematical Physics* and *Physics Letters*. This line of work is hardly one to engage the interest of the average civil servant. Nor, indeed, is his spartan lifestyle. Lord Avebury, the Liberal spokesman on immigrant problems, who is greatly interested in Dr Yoshimura's case told *Nature*: "The Home Office want everyone to fit into their procrustean rules. If you have rigid rules, there will always be cases where for reasons of humanity and common-sense, exceptions ought to be made. Dr Yoshimura's case is an example of this!"

US asked to reduce occupational radiation levels on basis of study of British dock-yard workers:

A Washington lobby group has asked President Carter to implement a ten-fold reduction in the permissible exposure to radiation of US workers on the basis of a British study of nuclear dock workers which has demonstrated that low level radiation can result in a significant increase in chromosomal damage (*Nature*, 15 February, p. 531).

In the letter, Dr Sidney Wolfe and Alexander Harris of the Health Research Group claim that almost five thousand workers in US atomic power plants were exposed to the levels of radiation that, in the British study, induced a four-fold increase in the incidence of damaged chromosomes over a ten-year period.

According to the group, the total radiation exposure in US atomic power plants increased 23% between 1976 and 1977, while the number of operating plants increased by only 8%, from 39% to 42%. Furthermore the number of workers exposed to more than 0.5 rem increased by 25% over this period to a total of 16,712. This is the level to which permitted exposure levels have been suggested should be reduced by Dr Edward Radford, chairman of the National Academy of Sciences committee on the Biological Effects of Ionising Radiation, whose own report is to be published within the next few weeks.

● The Nuclear Regulatory Commission last week ordered the temporary shutdown of five East Coast nuclear power plants following evidence that a "simple arithmetical error" in a computer formula may have resulted in cooling system pipes being made six times too weak to withstand the effects of an earthquake.

GMAG notice on self-cloning will exempt certain experiments:

Following a meeting on March 16, GMAG are expected to release a statement within the next two weeks on their requirement for notification of so-called 'self-cloning' experiments. Unsurprisingly, they have apparently decided to 'exempt' experiments in which genes from *Escherichia coli* are cloned in *E. coli*, genes from *Bacillus subtilis* in *B. subtilis*, and yeast genes in yeast. Those wishing to carry out such experiments will still be required to inform GMAG of their intention and will be expected to carry them out under conditions of 'good microbiological practice'. Also under consideration are proposals to handle in a similar way work with certain rigorously characterised recombinant DNA clones that have been agreed to carry no possible hazard.

MRC taken to High Court:

A further action has been started by solicitors for Dr Amiram Ur (21/28 December 1978) against the Medical Research Council. In addition to proceedings before the industrial tribunal for unfair dismissal a High Court writ served last week claims damages for breach of contract and wrongful dismissal, damages for losses due to negligence, breach of duty by the MRC in its conduct of Ur's application for tenured employment, and damages for "unlawful interference with the plaintiff's economic interests." The writ also names Professor I. M. Glynn of the Physiological Laboratory of Cambridge as co-defendant. Glynn was chairman of Ur's tenure committee.

Dr Amiram Ur's solicitors will now serve a statement of claim setting out in detail the allegations and the defendants will have to serve a defence. If the Court finds against the MRC damages could range from a payment of costs with a symbolic half penny award to the full amount claimed. Both the MRC and Professor Glynn had "no comment" to make on the action. Meanwhile the industrial tribunal reconvenes in London this week for another two weeks of hearings.

Geophysicists asked to write to Congress representatives to protect budget increases:

Members of the US geophysics community have been asked to spend six minutes working on letters to Congress representatives—urging them to support funds for basic science—for every hour spent preparing grant proposals, contracts and project justifications.

This suggestion of a commitment to "tithing" has been made by Professor Allan Cox, professor of geophysics at Stanford University and president of the American Geophysical Union. Writing in the union's newsletter, Professor Cox says that after several lean years of funding, the increase in funds for basic science promised by President Carter in his budget proposals for 1980 are necessary just to stay even.

"Moreover I would argue that to stay even we need to establish better communications with Congress", he says, pointing out that historically the basic research community—especially the academic part of it—has been "notoriously ineffective" in supporting its spokespersons in Washington. Such support is needed to protect the promised increases during the Congressional review process, he says.

According to Professor Cox, scientists are the only ones who can provide support for the administration's claim that their research is a good long-term national investment. "The pace of the budget process is such that a short letter now will be more useful than a long letter later."

Many members of the geophysics community were taken by surprise last year when Senator William Proxmire cut almost all funds for research into lunar samples brought back by the Apollo moon missions from the budget of the National Aeronautic and Space Administration.

US environmentalists provide some "good news" about energy: Adequate investment in energy saving techniques could allow the US economy to reduce its energy demand by 30-40%, and require only a 10-15% increase in energy usage over today's levels to maintain a healthy, expanding economy in the year 2000, according to a report published last week by the Council on Environmental Quality.

Techniques for increasing energy productivity include substituting heat pumps for electric resistance heating in homes, using greater insulation and more efficient appliances and machines in home and industry, and developing the use of total energy systems to generate heat and electricity simultaneously in commercial buildings, the report says.

"With a determined national effort to conserve energy, we can do well, indeed prosper, on much less energy than most people imagine," the chairman of CEQ, Mr Charles Warren, said on releasing the report, titled *"The Good News About Energy"*.

AAAS begins action against human rights violations in Argentina: The American Association for the Advancement of Science has decided to circulate widely its report and resolution on human rights violations in Argentina. The author of the report, Bruce Alan Kiernan, AAAS Human Rights Coordinator, observes that "Argentina continues systematically, in my view, to violate basic international standards of human rights: the right to security of person, to freedom from torture, to the right of trial and minimum standards of legal defense and appeal and humane standards of punishment". Of particular concern is the matter of disappeared persons, estimated by Kiernan to be between 20,000 and 30,000 people including many scientists—most of whom are believed dead. "40% of Argentine physicists have been kidnapped and some disciplines such as psychiatry no longer exist," says Kiernan. Senior advisors to President Videla admitted to Kiernan that many people in military custody were thrown out of helicopters, a tactic learned from American practice in Viet Nam. "I heard testimony of individual experiences in jails and detention centres and these accounts included descriptions of torture techniques that can only be described as horrendous." The AAAS resolution calls for scientific organisations to initiate on-site investigations in Argentina "on an urgent basis" and to release their results to their governments. The resolution also calls for the release and resettlement of falsely imprisoned Argentine scientists. The AAAS has asked the Human Rights Committee of the Organisation of American States to make a separate report on the condition of scientists. The AAAS Committee on Scientific Freedom and Responsibility regards the situation as "extremely bad" and has been meeting for the past week to consider among other things a call to scientists and scientific organisations to cut off all scientific exchanges to Argentina until the government restores human rights.

Friends of the Earth steps up campaign against Torness: The Torness Point nuclear reactor site in Scotland, the target for militant anti-nuclear demonstrations last year, is under new criticism from Friends of the Earth. On Wednesday, in what is described as "an eleventh hour effort to save us from yet another nuclear blunder" FOE

delivered a ten foot diameter millstone, the largest in Britain, to Bruce Millan, Secretary of State for Energy along with a copy of its report. FOE calls the government's outlay of £742m for Torness "an economic disaster twice the magnitude of Concorde". The report details numerous deficiencies in the government's proposal including: inadequate costing (a further £200-300m will be needed for the distribution system); inadequate need projections (the Scottish Electricity Board had a 65% surplus above maximum demand last winter); inadequate design on the two previous advanced gas cooled reactors; and loss of jobs (in the local community). *Torness: Keep it Green*. By Michael Flood. Friends of the Earth, 9 Poland Street, London W1V 3DG. 85p.



Shirley Williams proposes short term contracts for lecturers: In a speech on 5 March at The Times Higher Education Supplement's Conference on Trends in Education into the 1990s, Shirley Williams, Secretary of State for Education and Science suggested that short term contracts for university and polytechnic lecturers may become a necessity. Basing her argument on demographic considerations,

Williams predicted that a current "hump" in student enrolment would peak in the universities in the mid-1980s. Half the number of lecturers required to teach this excess above "background" could then be expected to be employed on short term appointments. In addition, Williams feels that short term contracts could provide room for young lecturers and for dismissal of older lecturers who had not fulfilled their "early promise". Laurie Sapper, General Secretary of the Association of University Teachers said that Williams' proposal was "just a disguised way of saying that the universities should employ cheap labour".

US report says no solution yet to nuclear waste disposal: An interagency working group has told President Carter that there is still insufficient evidence to assess the feasibility of safely disposing of nuclear waste in underground repositories, and that such evidence could only be acquired by investigating the suitability of particular sites.

The group, which was set up by President Carter last year, said that current knowledge was adequate only to identify possible storage sites for further investigations. It also said that conservative engineering judgment, as well as providing multiple barriers between radioactive material and the environment, could reduce some of the risks involved and compensate for some of the uncertainty.

West German fast breeder report criticised: Dr Ulrich Steger, chief spokesman of the SPD working group for research and technology in the *Bundestag*, has fiercely criticised a recent report on the future of the fast breeder in Germany. The report, by Dr August Eitz of the Schnell-Brüter-Kernkraftwerkgesellschaft (SBK), deplores the delay in licensing the prototype SNR-300 fast breeder at Kalkar on the Dutch-German border, minimises the risk of accidents, and argues that nuclear proliferation is unavoidable except by international surveillance. The use of nuclear energy leads logically to fast breeders to conserve uranium stocks, Eitz says. But according to Steger, Eitz—in order to put the breeder in a favourable light—has offered "only stale growth-fetishism". According to Steger anyone looking for the reasons why nuclear energy is in a difficult situation would find some of them in Eitz's speech.

correspondence

INFCE looks for technical and institutional solutions

SIR,—I would like to make some corrections to the report entitled "INFCE halfway through" (8 March, page 112). Although the idea of INFCE was first suggested at the London Summit, it was set up in Washington in October 1977 and involves not only the Western industrialised nations but the Soviet Union, several of the East European States and quite a number of developing countries. It is incidentally organised into eight working groups, not twelve.

At the time INFCE started, the proliferation risks of reprocessing were indeed judged differently by on the one hand the United States and Canada and on the other all the major nuclear power users (not simply the UK as your article suggests). These countries all felt that the case for reprocessing must take into account environmental and economic factors; and that as spent fuel contained plutonium, spent fuel storage also carried proliferation risks. However, as a result of studies in INFCE it is now apparent that some intermediate fuel storage is necessary because it is most unlikely that world reprocessing capacity will be sufficient to deal with all spent fuel arising for perhaps another 20 years, and some spent fuel will therefore have to be stored for a considerable time.

Your article is correct in implying that there is no simple technical fix to the proliferation risks arising from the growing spread of civil nuclear power. But we are also engaged in an examination of institutional measures to counter the dangers of proliferation. The International Atomic Energy Agency has already shown itself to be an eminently valuable institution, especially its safeguards system, and it may well be that internationally controlled plutonium storage and internationally controlled spent fuel storage can be important further additions to world confidence.

Yours faithfully,
SIR HERMANN BONDI

Chief Scientist,
Department of Energy, London.

Defence R&D and SRC budgets are not comparable

SIR,—I was interested to see your editorial (1 March, page 1). Most members of the scientific community in the UK will agree with you that the "difficult question"—the minimum rate at which expenditure by the research councils should grow so as to sustain the health of research in the country—should be addressed with urgency. It is good to see a lead from the ABRC, for it is not only sustenance that is at stake; there is a vital need to repair the attritions of the past decade or more.

I regret, however, that you should have confused this important problem with a less than adequate examination of the relativities of government R&D resource allocation. The defence R&D budget and SRC expenditure set out to do quite different things: SRC remains, I believe, dedicated to the promotion of research of 'timeliness and promise', and the research councils accept responsibility—as they must—for the nurturing of the research function in universities, polytechnics and their own laboratories. Less than 1% of defence R&D expenditure can be identified with the support of innovative research: a matter of considerable concern to myself and my colleagues here. As the 1979 Defence White Paper—or the more recent Review of Cmnd 5046—indicates, more than 70% is invested in British industry (including, for instance, the important area of electronics) and is dedicated to the short term task of developing new equipment essential to the Armed Forces and national defence. Thus about 25% of the R&D budget finds its way to the defence establishments, and this too is overwhelmingly dedicated to short and medium term development work.

Moreover, I fear that your misunderstanding of the equivalence (or lack of it) between defence R&D and the SRC budgets reads over to your remarks on the competence of personnel in our R&D establishments. It will be clear from the above that the criteria for judging the ability and performance of scientists and engineers in defence laboratories must, in general, be quite different from those applied to my colleagues in the universities. This is not to say, of course, that MOD scientists inhabit a world isolated from the wider scientific community. We are always looking for ways of promoting contact; but communication is already frequent and fruitful, much of it through the Defence Scientific Advisory Council, which monitors very carefully the programmes of all R&D laboratories and which submits independent annual reports to the Secretary of State. There exist other forms of "peer review", internal and external, both for programmes and for individual scientists and engineers.

There may, in fact, be less complacency in defence about the need to repair the innovative function than there is elsewhere. The R&D establishments have suffered considerable erosion for a number of years, during which their work has been directed away from the long term innovative task. It would be unfair to attack them now on the basis of a spurious comparison of their contribution to microbiology or solid state physics, or whatever, with those emerging from research sponsored by the research councils. The latter are conducted for different purposes, and are different in kind.

Yours faithfully,
RONALD MASON

Chief Scientific Adviser,
Ministry of Defence, London.

Piltown stains

SIR,—In your correspondence columns (22 February, page 596) Dr L. B. Halstead recalls that he refuted my suggestion (*The Times*, 7 November 1978) that Sollas may have used potassium bichromate to harden fossils to which he applied his serial sectioning technique. I wrote a letter in reply, but it was not published owing to the temporary cessation of *The Times*. I accept the validity of the evidence which Halstead found in the records of the University Museum, Oxford, showing that all the fossils to which Sollas applied serial sectioning were hard by nature, and therefore required no artificial hardening. The truth of the matter is that after the passage of some 70 years there is nothing to be gained by speculating on the purpose for which Sollas ordered the packet of bichromate crystals. As I pointed out in my letter to *The Times* of 7 November, Charles Dawson himself stated that he soaked some of the Piltown bones in a solution of potassium bichromate, allegedly for the purpose of hardening them. Only by special pleading could anyone argue that this chemical was ordered by Sollas for delivery to him in Oxford, after which (one would have to suppose) it was forwarded to Dawson in Lewes with instructions as to how it might be used. These are the thoughts of a Jabberwocky! Dawson would have had no difficulty in obtaining potassium bichromate from a pharmacy in Sussex.

Researches by Dr (now Sir Frank) Claringbull and Dr M. H. Hey at the British Museum (Natural History) (Further Contributions to the Solution of the Piltown Problem, *Bulletin of the British Museum (Nat. Hist.)*, Geology series, vol 2, no 6 (1955)) showed that the Piltown cranial bones had been artificially stained by treatment with a solution of an iron sulphate. The additional use of a bichromate solution can now be understood because it would serve to aid the oxidation of a ferrous iron salt and thus the production of rusty staining. So the bones, which were originally of pale biscuit colour, in the end matched the dark reddish-brown Piltown gravel in which they were supposed to have been found.

Dawson is known to have carried out experiments on the staining of bones and flints. He may well have required advice from somebody with greater knowledge of chemistry than he himself possessed, but it is unlikely that he would have sought help from the Oxford Professor of Geology. He was in a position to obtain chemical advice locally: in his first paper on Piltown, read to the Geological Society in December 1912, Dawson thanks Mr S. A. Woodhead, Public Analyst of East Sussex, for chemically testing a piece of the Piltown cranium and reporting that no organic matter could be detected in it (recent analyses of the cranial bones showed protein residues represented by 0.2–1.9% nitrogen).

Yours faithfully,
KENNETH P. OAKLEY

Oxford, UK

news and views

Aetiology and genetic control of natural scrapie

from Richard H. Kimberlin

SCRAPIE disease, a neurological disease found naturally in sheep, is generally considered to be caused by a transmissible agent. Recently, however, Parry¹, on the basis of studies of the incidence of natural scrapie in commercial flocks has reiterated his long-held belief^{2,3} that scrapie is a disease solely of genetic origin, controlled by an autosomal recessive gene. Although Parry does not dispute the existence of a transmissible scrapie agent he believes it to be "formed *de novo* in each affected animal by the metabolic activity of the natural recessive gene", and considers it irrelevant to the disease, or at least not involved in its spread.

In view of his comments it may be useful to re-examine the role of the transmissible agent in scrapie. Many scientists in the field consider that scrapie is caused by a transmissible agent, with host genetic factors exerting some kind of control on the development of clinical disease. In my opinion this interpretation is not only supported strongly by available data but it does not conflict in any way with Parry's interesting results.

Evidence for a transmissible agent

There is no doubt that the injection of brain homogenates from affected sheep will transmit the disease to other sheep after long incubation periods⁴. The transmissible agent can be filtered^{5,6} and experimentally passaged in sheep to extremely high dilutions of original inoculum⁷, thus showing the presence of a replicating, virus-like agent. Experimental forms of scrapie have been produced in many species⁸, mice in particular, and it is notable that mouse-passaged agent can also be injected into sheep to produce scrapie^{9,10}. This evidence alone provides a substantial reason for concluding that the transmissible agent causes the natural disease.

Many different strains of scrapie agent have been isolated in mice from natural sources⁴ and it is now known that some of these strains undergo mutation on serial passage¹¹. Strain

variation and mutation are the expected attributes of a pathogenic microbial agent. There is a serious problem with Parry's suggestion "that the scrapie TSEPA [transmissible spongiform encephalopathic agent] is formed *de novo* in each affected animal by the metabolic activity of the natural recessive gene" because one has to explain how a single host gene can produce biologically different strains of agent, sometimes in the same animal¹.

However, the main issue raised by Parry is not the existence of the scrapie agent but whether or not it is naturally infectious. I would like to summarise three major studies that strongly indicate natural spread of agent from one animal to another.

In one experiment¹², 11 Blackface sheep were close-penned from birth until 3 to 4 years of age with a succession of naturally affected sheep. Four died of intercurrent disease before they reached an age at which scrapie first occurs, but three of the remaining seven developed scrapie. In the same study¹², 17 goats were exposed under similar conditions and 10 of these developed scrapie. As considerable care was taken to ensure that the exposed animals were obtained from scrapie-free stock the high proportion of scrapie cases in Blackface

sheep (43%) and in goats (59%) strongly suggests contagious spread.

In a second experiment¹³, the exposed Scottish Blackface sheep came from a well documented flock in which no cases of scrapie had been seen in over 18,000 sheep during 13 years. However, when some of these Blackface sheep were kept in life long contact with a Suffolk flock with a high incidence of natural scrapie, and managed under normal commercial conditions, 21 (28%) of the exposed sheep developed scrapie. It is extremely difficult to explain such a high level of scrapie in the Blackface sheep except by contact spread of agent.

Even more impressive data have been obtained from the USA with Angora, Nubian and Toggenburg goats and Rambouillet, Targhee, Hampshire and Suffolk sheep, all from scrapie-free flocks. It should be noted that no field cases of scrapie have been found in Angora goats or in Rambouillet or Targhee sheep in the USA. Yet when these and the other animals (total 140) were put in contact with scrapie-affected flocks, five developed the disease¹⁴. These 140 animals were bred within their own groups in the scrapie environment and produced 358 progeny that survived for at least two years. Of these, 98 (27%) developed scrapie. The figures for individual

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17. Dickinson, A. G., Young, G. B. & Renwick, C. C. *ibid.*
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22. Dickinson, A. G. & Outram, G. W. in *Slow Transmissible Diseases of the Nervous System* (eds Hadlow, W. J. & Prusiner, S. B.) in the press (Academic Press, New York).

breeds ranged from 14 to 39% for sheep and from 26 to 61% in goats¹⁴. Again, the conclusion that contact spread of scrapie occurred seems inescapable.

Genetic control

The evidence that scrapie is an infectious disease is clearly incompatible with a disease of genetic origin. However there is no reason why genetic factors in the host should not control the process of infection or the development of disease once infection has taken place and this was the conclusion drawn from other studies of genetic factors in natural scrapie^{4,13}.

There are three major studies showing that the probability of a lamb's developing scrapie depends more on the eventual scrapie status of the ewe than of the ram¹³⁻¹⁵. These observations of maternal bias indicate transmission of infectious agent from ewe to lamb and there is evidence from other sources that agent is probably passed to the embryo^{4,16,17}. However the extent of this maternal bias will depend on many factors, not least of which is the level of contagious spread of agent, which if relatively high may obscure maternal transmission. Hence the fact that Parry finds no evidence for maternal transmission of agent may simply mean that in some situations it is not of paramount importance.

The only other area where there are conflicting data concerns the nature of the genetic control exerted by the host on the development of disease. Parry's results¹ indicate that the natural disease in his flocks is controlled by an autosomal recessive gene whereas another study¹³ showed a more complex and unresolved genetic control of natural scrapie. Two further investigations^{18,19} demonstrated that the development of clinical scrapie in Cheviot and Herdwick sheep following injection with the SSBP/1 source of scrapie agent was mainly controlled by a single gene with the fully dominant allele conferring susceptibility. A broadly similar genetic control of the natural disease in these two flocks is indicated by the subsequent appearance of natural scrapie which is largely confined to the susceptible lines^{4,20}. This diversity of findings cannot easily be explained at the moment but the following observations may be relevant.

First, given that scrapie is caused by an infectious agent, then variables such as the strain of agent, the relative importance of maternal and contagious spread of infection and the age of the animal when exposed, could conceivably involve different genetic components.

Second, the genetic studies of experimental scrapie in Cheviot¹⁸ and Herdwick¹⁹ sheep used the same source

of scrapie agent and gave similar results. However it has now been shown that the response of genetically selected lines of Cheviot sheep to an agent designated CH1641 is quite different from that found with SSBP/1 (refs 4,10). This means that the genetic control of experimental sheep scrapie varies with the strain of agent.

The third point follows from this and concerns the interaction seen between different strains of mouse-passaged scrapie agent and the murine gene *sinc* which controls incubation period^{21,22}. The gene has two alleles, *s7* and *p7*. Many agent strains (for example) ME7, 22C, 139A) have a shorter incubation time in mice homozygous for *sinc*^{s7} than in *sinc*^{p7} mice, but agents such as 22A have the reverse properties. When comparisons are made of incubation periods in *s7p7* mice, the interactions between agents and the alleles of the *sinc* gene range from no dominance or partial dominance of one or other allele, to full dominance and even positive overdominance in which the incubation period is longer in the heterozygote than in either of the homozygotes^{21,22}.

If this level of complexity applies to the natural disease then it would be quite wrong to expect the genetic control of scrapie to be the same in all situations and the fact that different studies have given different results may be a reflection of this. □

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Conversion of mouse fibroblasts into adipocytes

from Franklin H. Portugal

AN important new avenue of research on lipid metabolism and obesity has been opened up through the conversion of mouse fibroblasts into adipocytes under defined culture conditions. In 1974, Green and Kehinde (*Cell* **1**, 113; 1974) observed a few cells with lipid droplets in resting cultures of 3T3 mouse fibroblasts. They suggested that these observations indicated the establishment of clonal lines of fibroblasts that have a high frequency of conversion into adipocytes when their growth is arrested. Because low concentrations of bromodeoxyuridine block this conversion without affecting other specialised cell functions such as collagen synthesis, the conversion pro-

cess involves differentiation of the fibroblast.

In contrast to the fibroblast, the adipocyte acquires the signet ring appearance of adipose cells, accumulates triglycerides, and loses the ability to revert to the growing state. Insulin, L-adrenaline, N⁶, O²-dibutyryl cyclic AMP and isoproterenol alter the incorporation of precursors into triglycerides in the adipocytes. During the conversion from fibroblast to adipocyte, there is a 40-50-fold increase in the incorporation of ¹⁴C-acetate into triglycerides. This increase is paralleled by an increase in lipogenic enzyme activities including acetyl-CoA carboxylase, ATP-citrate lyase, and fatty acid synthetase (Mackall *et al. J. biol. Chem.* **251**, 6462; 1976). For one of these enzymes, acetyl-CoA carboxylase, the increase in activity is due to an increase in the cell enzyme level as determined by measuring the change in immunotitratable enzyme. Lipoprotein lipase, a lipolytic enzyme, also increases rapidly during the post-confluent period (Eckel *et al. Biochem. biophys. Res. Commun.* **79**, 720; 1977). The conversion of 3T3 fibroblasts to adipocytes is also sensitive to agents that affect cyclic nucleotide levels, such as prostaglandin F₂ and 1-methyl-3-isobutyl xanthine (Russell & Ho *Proc. natn. Acad. Sci. U.S.A.* **73**, 4516; 1976). Adenylate cyclase of adipocytes compared with the enzyme in preadipocytes is markedly stimulated by isoproterenol and shows a response to adrenocorticotrophic hormone (Rubin *et al. J. biol. Chem.* **252**, 3554; 1977). Near-physiological levels of insulin stimulate the conversion of glucose into carbon dioxide and lipid only in the adipocyte.

Serum factors also seem to play a part in the conversion of fibroblasts. Recently, Kuri-Harcuch *et al. (Cell* **14**, 53; 1978) reported that in serum-supplemented medium, 3T3 fibroblasts can still apparently differentiate into adipocytes even in the absence of biotin which is essential for fatty acid synthesis. They became spherical, the activity of NAD-linked glycerolphosphate dehydrogenase increased 300-fold and the activity of lipoprotein lipase increased 50-fold. But without biotin the cells were unable to increase the rate of acetate incorporation into triglycerides.

Subsequently Kuri-Harcuch and Green (*Proc. natn. Acad. Sci. U.S.A.* **75**, 6107; 1978) have identified a non-lipid adipogenic factor in serum. In the presence of this factor, cells grown to confluence become spherical, increase their malic enzyme and glycerolphosphate acyltransferase activities, and accumulate triglycerides, whereas in its absence none of this occurs. Fetal calf serum is much richer in this factor than calf serum or the other

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mammalian sera that were tested. This serum factor differs from biotin in that it regulates cell shape and differentiation of cell enzymes.

Négrel *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 6054; 1978) now report that they have established a clonal line of adipose cells from epididymal fat pad cells of genetically obese C57BL/6J *ob/ob* mice. At the resting stage, these converted fibroblasts accumulate triglycerides. Differentiation of these cells is accompanied by increased lipoprotein lipase, acid:CoA ligase, and acyl-CoA:diglyceride acyltransferase activity. They respond to hormones including adrenaline, corticotropin, and insulin. Bromodeoxyuridine almost totally prevents the presumed induction of the triglyceride-pathway enzymes. Conversion of *ob* 17 cells to adipocytes apparently differs in several respects from the conversion of 3T3 cells obtained from the Swiss mouse embryo. These differences may include sensitivity to insulin and biotin as well as lower values for fatty acid biosynthesis.

The fact that these adipocyte cells in culture show many of the same characteristics as adipocytes *in vivo* suggests that they can be useful models

for understanding the factors that control obesity. Comparisons of adipocytes from genetically and non-genetically obese mice may help to clarify the genetic factors that regulate obesity. Furthermore, studies of lipid metabolism that are difficult with intact animals are now simplified. For example, the use of adipocytes in culture suggests that the appearance of lipoprotein lipase is not substrate-induced by the presence of serum triglycerides or lipoproteins. These cells are also important for distinguishing true lipogenic enzymes such as pyruvate carboxylase from lipogenic-type enzymes such as aldolase. An understanding of how fibroblasts are converted into adipocytes should also increase our understanding of the mechanisms that control cell differentiation. In addition, these cells can increase their number of insulin receptors whereas most systems show either no change in response to increased insulin levels or decrease their number of insulin receptors. Thus, the conversion of fibroblasts into adipocytes will also be useful for determining the factors controlling receptor development. □

mean atomic weight is effectively constant to within 2%, and Shankland (*Geophys. Surv.* **3**, 69; 1977) has suggested on the basis of a study of chemical and crystallographic properties of supposed mantle materials that "the mantle appears to be chemically homogeneous". Above 700 km the mean atomic weight is apparently more variable because of the occurrence of phase transitions at several depths; but Schubert and Turcotte (*J. geophys. Res.* **76**, 1424; 1971) have already shown that convection is not thereby prohibited. In summary, then, there appears to be no significant reason why convection should not occur anywhere in the mantle.

The case for whole-mantle convection as such was recently boosted by the detailed studies of Davies (*Geophys. J.* **49**, 459; 1977) who used the arguments quoted above, among others. And it has now been given further support by Elsasser *et al.* (*J. geophys. Res.* **84**, 147; 1979) who have set the scene for the decision that must ultimately be made between, on the one hand, convection down to 700 km only, and, on the other, convection down to the core-mantle interface. As Elsasser and his colleagues point out, the "entire question of mantle convection has lately entered a new stage" in view of the conclusion by Rochester *et al.* (*Geophys. J.* **43**, 661; 1975) that precession provides too little power to drive the core dynamo, which must therefore be driven either by radioactivity-induced thermal convection or by the progressive falling out of Fe and Ni at the boundary of the inner core.

It follows from this (cutting out many stages of the argument) that, if radioactive heat transfer in the mantle is small (as is widely supposed), there must be a hot thermal boundary layer at the base of the mantle, for the quantity of heat leaving the core will be greater than the mantle can dissipate by conduction alone. The build-up of heat that this implies will then result in the detaching and upward motion of parts of the boundary layer material, thereby setting up a system of motion in the mantle. From this starting point, and the deduction that the temperature at the core-mantle interface is $4,000 \pm 500$ K, Elsasser *et al.* go on to develop a model of whole-mantle convection that works in the sense that, for all its assumptions, it is consistent with global heat flow, plate sizes, plate speeds, mantle viscosity and certain seismic data.

The picture thus conjured up is one of extreme simplicity—whole-mantle convection moves continents and sea floors and leads to surface volcanism whilst whole-(outer)core convection generates the lower boundary condi-

Mantle convection: shallow or deep?

from Peter J. Smith

A QUESTION that has exercised the minds of geophysicists on and off for more than 40 years now, is that of the vertical scale of convection in the mantle, assuming that convection takes place there at all. Thoughts on this subject may be divided conveniently into three periods. During phase 1 the pioneers (Vening, Meinesz, Holmes, Runcorn for example) evidently took it for granted that convection would be mantle deep, a view that prevailed generally (at least among the supporters of continental drift) up to the early 1960s. But the rapidly growing appreciation of the special properties of the asthenosphere—in particular its relatively low viscosity—gradually convinced many, but not all, that convection limited to the upper mantle was a viable alternative. Not that convection was regarded as necessarily being limited to the asthenosphere itself—that is, to a maximum depth of about 250 km. Some took the view that 700 km or so should be regarded as a lower limit because earthquakes occur to that depth, largely in subduction zones where lithosphere is descending. Either way, however, it

did not seem unreasonable to suppose that convection stopped well short of the core-mantle boundary at 2,900 km. That was phase 2.

Phase 3 began sometime during the past two or three years, marking a period of maturity during which both shallow and deep convection hypotheses must battle it out on more equal terms.

For example, by the late 1960s a number of geophysicists had concluded that below the asthenosphere the viscosity increases rapidly with depth in the mantle, thereby, it was suggested, making convection in the deeper mantle impossible. But ideas on that have now changed, largely as the result of the work of Cathles (*The Viscosity of the Earth's Mantle*, Princeton University Press, 1975) and Peltier (*Geophys. J.* **46**, 669; 1976) who have made convincing cases (by different routes) for a mantle viscosity more or less constant at about 10^{21} poises below the asthenosphere.

Another condition that would preclude whole-mantle convection would be a variation of gross chemical composition with depth. But Watt *et al.* (*Geology* **3**, 91; 1975) have concluded from free oscillation data that between 700 km and 2,900 km in the mantle the

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tions for mantle convection as well as the geomagnetic field. Moreover, as volcanism has evidently been occurring and the geomagnetic field has evidently existed for at least 3×10^9 years and probably getting on for 4×10^9 years, the convective systems would appear to be almost as old as the Earth. As Elsasser and his coworkers concede, "this is in line with Tozer's often expressed view that since planets are convectively unstable and since the radioactive heat of their interiors can only be removed by convection (for example, Tozer *Phys. Earth Planet. Int.* 6, 182: 1972), most planets can be expected to convect most of the time".

Obviously an excellent case can be made for whole-mantle convection. Whether it actually occurs or not is more difficult to prove. □

Reproduction in flowering plants

from A. J. Richards

INTRODUCING the International Symposium on the Reproduction of Flowering Plants, held recently in New Zealand*, E. J. Godley (DSIR, Christchurch) discussed the special characteristics of the New Zealand flora. One question posed was whether the proportion of unisexual flowers found in New Zealand is as uncommon as has been previously supposed. Twelve per cent of the 1,800 species of indigenous flowering plants are dioecious (with stamens and pistils on different plants) compared with 2% of indigenous UK species. However, high frequencies of unisexual plants are common in tropical forests (as shown in Costa Rica recently by K. S. Bawa (University of Massachusetts)) and are found in all temperate countries south of the Equator. Dioecy is generally regarded as being a secondary phenomenon, hermaphrodite flowers being the primitive type in the flowering plants, so it seems unlikely that dioecy is a hang-over from the ancient southern continent of Gondwanaland. Perhaps the high level of variability and vigour engendered in outbreeders was favoured during the establishment phases of the southern land-masses, as they became increasingly isolated from the mainstream of flowering plant evolution to the north.

Godley also discussed the predominance of white and yellow flowers in

New Zealand, so well-known in the hebes, olearias and celmisias cultivated in the UK. He quoted Cockayne, stating that of attractive flowers, over 60% are predominantly white flowered in New Zealand (the comparable figure for the UK is 25%). Certainly, the experience of a casual visitor from Europe, faced with white gentians, white buttercups, white willowherbs and white forget-me-nots bears this out. Godley thinks that white flowers, mostly of an unspecialised nature, might also be representative of an ancient, isolated southern continent, and many anthecologists would agree that this simple syndrome ties in well with the paucity of pollinators that a land-mass, isolated so early, might be expected to demonstrate. Specialisation of flowers will only succeed if a rich flower-visiting fauna is present.

Until recently, gynodioecy, in which pistillate and hermaphrodite plants are mixed, was thought to be uncommon. A number of workers showed that it is much more frequent than previously realised. Theoretically, it is difficult to model a balanced gynodioecy; either the disadvantage to pistillate flowers of requiring cross-pollination for seed to set, and having fewer potential pollen sources; or the advantage of not bearing the reproductive cost of stamens, is likely to upset the balance. This is compounded by the tendency for male sterile genes to be carried cytoplasmically, and thus to be inherited maternally. It was suggested that in stable gynodioecious conditions, nuclear-controlled male fertility restorers help natural selection in the maintenance of a balance between male fertile and male sterile plants. Marianne Philipp (University of Copenhagen) provided some interesting data to show how this balance might operate selectively.

X. Delanny (University of Louvain) echoed the work of other speakers in showing just how cryptic gynodioecy can be. Most European species of thistle are gynodioecious, but the over-familiar creeping thistle, *Cirsium arvense*, is usually fully dioecious although morphologically the flowers appear hermaphrodite. Females show early abortion of pollen, despite normal looking anthers, and males have perfectly functional pistils, apart from the pollen-receptive papillae on the stigma, which fail to develop.

As in so many other sciences, reproductive genetics seems to be emerging with difficulty from a model-building phase, and there are still more ideas than facts. However, this is much less true with respect to recent work on the physiology of sexual fusion in flowering plants. Using technically outstanding light and electron micrographs

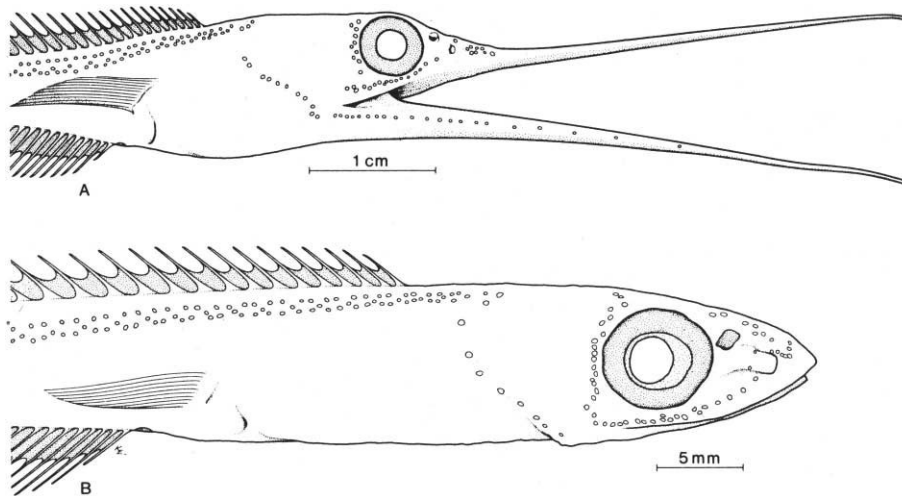
of cotton ovules W. A. Jensen (University of California, Berkeley) has followed the fate of the male gamete immediately before fertilisation in fascinating detail. The generative nucleus of the pollen tube is a true cell, surrounded by a plasma membrane. On entering the embryo-sac, the pollen tube forms an association with one of the synergid nuclei. This synergid grows and migrates to the egg nucleus, carrying the male cell which has been discharged by the tube into the synergid. Plasma membrane between the male cell and the egg are sequentially broken down, finally permitting controlled fusion. The remains of the synergid subsequently throws out the second male nucleus towards the fused polar nucleus (with which it will fuse to form the triploid endosperm). The polar dikaryon has already fused on the stimulus of pollination, and this fusion is probably triggered by secondarily produced gibberellic acids. In a similar way, the growth and change of appearance of the receptive synergid is apparently triggered hormonally, and can be induced by auxin placed on the stigma in the absence of pollen. There is now good evidence that the directional chemotaxis of the pollen tube occurs in response to a calcium gradient, according to evidence achieved with the use of ion-analysing microprobes.

Ultrastructural work has also thrown light on the control of apospory, a form of agamospermy (seeds without sex) in the New Zealand grass *Cordalaria*. Melva Philipson (DSIR, Christchurch), showed that sexual meiosis is able to proceed towards the formation of megaspores and an embryo-sac, protected by a callose sheath. In aposporous conditions, the callose breaks down early, and the megaspore mother cell degenerates, allowing the formation of vegetatively budded, maternal embryo-sacs, which develop embryos autonomously, identical to the parent.

There was much more to report at this conference: a mass of exciting anthecological and breeding system work is emerging from the botanical wealth of Australia, which with over 20,000 species of flowering plants, and a host more waiting to be described, bids fair to replace California as the evolutionary field laboratory of the world. Exciting new techniques were also in evidence, especially in the fields of cell fusion studies, somatic gene transmission, embryology, using new clearing techniques to replace tedious sectioning, and the use of artificial dried 'seeds' cloned from tissue cultures. □

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*The International Symposium on Reproduction in Flowering Plants, organised by the Royal Society of New Zealand was held on 5-10 February, 1979 at the University of Canterbury.



Head of *Nemichthys scolopaceus*: A, ♀; B, ripe ♂.

Snipe-eels unravelled

from a Correspondent

THE snipe-eels are a family of eels found in mid-water in deep sea throughout all the temperate and tropical oceans. They derive their common name from the extraordinary elongation of the jaws which resemble a snipe's bill, except that the tips curve outwards in the vertical plane. In common with almost all groups of eels there has been much confusion in the nomenclature and systematic arrangement of the group although for several reasons the snipe-eels have been exceptionally complicated. A recent study by J. G. Nielsen and D. G. Smith on the eel family Nemichthyidae (*Dana-Report* No. 88, 1; 1978) will, however, go a long way to clearing up the confusion in these fishes.

One of the problems with these eels is that they live in mid-water and until the development of effective mid-water nets in the middle of the present century, most of the specimens seen by naturalists were accidental captures made while hauling bottom trawls. Indeed, it is not infrequent for deep-water fishermen to find snipe-eels entangled by their slender 'beaks' in the wings of a bottom trawl. Most of the specimens described by early ichthyologists were thus isolated captures and often not in good condition (*Nemichthys scolopaceus* grows up to 1 m in length, is about 2 cm deep at its deepest part, and less than 1 cm wide—so general fragility might be expected). Another potent source of confusion arose from this fragility, such as when one species was described as new to science on the grounds that there was a ridge of skin on the dorsal surface of the snout—a feature which Nielsen and Smith show to be due to shrinkage of the skin. These and other factors all lead to a thorough con-

fusion within the family, and it seems from the historical account of Nielsen and Smith that the group was fated to attract workers who failed to appreciate critical differences and even similarities. Since 1848, when the first genus *Nemichthys* was described, no fewer than eleven other genera have been proposed in the family, and the plurality of species has been even greater. In the current revision this has been reduced to three genera; only nine species are recognised.

A major factor in the artificial boost in the number of species was that mature male snipe-eels look totally dissimilar to females and immature fish. This sexual dimorphism was demonstrated by Smith and Nielsen (*Steenstrupia* 4 (1), 1; 1976) in a preliminary note, but the phenomenon is discussed in greater detail in their current revision. At sexual maturity male snipe-eels lose the characteristic snipe-bill, lose their teeth in the process, develop tubular anterior nostrils, change colour, and their pectoral fins move posteriorwards. Sexually mature females may also change colour and partially lose their teeth. So great are the differences between males and females that for over 50 years they have been placed in separate genera, and by some workers even in separate families and suborders!

It seems probable that the males at least spawn once and die soon after. The scarcity of short-nosed snipe-eels in collections suggests that they are rare in nature. However, as Nielsen and Smith point out, fishing gear may select in favour of the females, which having teeth and long snouts are more likely to become tangled in netting than are the toothless, short-snouted males. No doubt their study will stimulate further investigation of the ratio of male to female snipe-eels.

The fascinating shape of the jaws is briefly discussed but no new light is

shed on the means by which these fish capture their food, which is mainly crustacean. An earlier suggestion by G. W. Mead and S. A. Earle (*Proc. Calif. Acad. Sci.* 38 (5), 99; 1970) was that the long, tooth-studded non-occlusible jaws might be used to entangle the antennae of the sergested and hoplophorid shrimps which they eat. The backward-pointing teeth would ensure that any trapped animal could only move towards the gullet, and yet the awkward divergence of the upper and lower jaws hardly seems to be an efficient method of capturing food.

Nielsen and Smith's study is a major advance in clarifying the confusion in taxonomy of the snipe-eels, but it is unfortunate that it appeared at almost the same time as the publication of a study by E. S. Karmowskaya (*Trudy Inst. Okeanol.* 109, 186-210; 1977) on the taxonomy and distribution of the genus *Borodinula*, with the description of a new species. Nielsen and Smith refer to this paper in a postscript added in proof, pointing out that Karmowskaya's new species is a species they consider was described as long ago as 1916, while *Borodinula* is a synonym of *Avocetina*. Evidently the era of confusion in snipe-eels is not yet over.

Equatorial upwelling rates

from M. Tomczak Jr.

ESTIMATES of vertical velocities in the ocean have long been a matter of interest in oceanic science since the supply of nutrients for the surface layer where primary production through photosynthesis can take place, almost exclusively depends on renewal from below. Apart from the important coastal upwelling regions along the coasts of California, Peru, North-west and South-west Africa, there is clear evidence for strong upwelling caused by a divergence in the surface layer flow along the Equator. To estimate the productivity of the region, however, is difficult because upwelling rates, that is, the amount of water which upwells into the surface layer per unit time interval cannot at present be measured directly. Average vertical velocities are believed to be of the order of a few centimetres per day or less.

Theoretical estimates of upwelling rates are usually based on the calculation of 'Ekman layer suction', the amount of water absorbed into the surface layer from below in order to compensate for local losses due to divergences in the surface currents. Since surface currents are wind-driven it is possible to calculate this effect

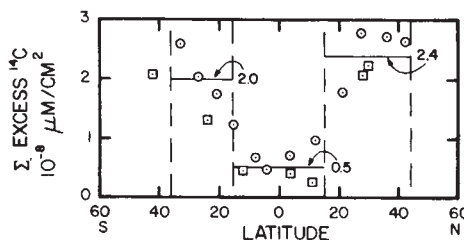


Fig. 1 Radiocarbon content of the Atlantic Ocean, integrated over the water column. The dashed lines define the equatorial and temperate zones in the study of Broecker, Peng and Stuiver (*op. cit.*) from which the figure is taken.

from data on the wind stress distribution over the ocean and some effort has been devoted to a more detailed knowledge of this important parameter (Leetma & Bunker *J. mar. Res.* **36**, 311; 1978). Application of these considerations to the equatorial zone leads to upwelling rates of $15\text{--}20 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ for the Atlantic Ocean which means that the upward flux of water into the surface layer, distributed over the entire Equatorial Atlantic Ocean, amounts to about one quarter of the transport of the Gulf Stream along the American coast. In a recent paper by Broecker, Peng and Stuiver (*J. geophys. Res.* **83**, 6179; 1978) the same order of magnitude is derived from the distribution of radiocarbon which entered the ocean after the bomb tests between 1955 and

1965. Their result is of particular interest because it represents a method of assessing upwelling rates which is completely independent of any meteorological parameter and, if it can be accepted as reliable, could provide a valuable reference value for other calculations.

Broecker, Peng and Stuiver start from the fact that the radiocarbon content of the Equatorial Atlantic Ocean is low by a factor of about 4–5 compared with the temperate zones and proceed to consider a number of models on the assumption that the water of the surface layer in the equatorial zone is removed sideways into the temperate zones and replaced by upwelling. In the simplest of their models the equatorial zone is treated as a well-mixed homogeneous layer of 150 m depth between 15°N and 15°S . The choice of the lateral boundaries seems natural from the observed bomb radiocarbon distribution (see Fig. 1). In the vertical, other models use a succession of layers with a 100 m top layer and three 50 m layers below. The resulting upwelling rates vary between 15.6×10^6 and $20.2 \times 10^6 \text{ m}^3 \text{ s}^{-1}$.

Encouraging as these results might seem, the problem with them is that the models could well be called an oversimplification of the actual situation. It is known and has been reported in *Nature* by Düing *et al.* (257, 280; 1975) in a review of the GATE Equa-

torial Oceanographic Experiment that the equatorial current system between 15°N and 15°S does not display a simple homogeneous structure (Fig. 2). Broecker, Peng and Stuiver acknowledge the fact and state that their results should be taken as averages over the entire area. It might well be questioned whether an average over two westerly currents and three easterly ones has any significance. More important is the neglect of all zonal transport in the model. The authors calculate, on the basis of their upwelling rate, a vertical velocity of about 30 m yr^{-1} and a residence time of 4.7 yr for the water in the surface layer. Now, even a modest flow of 25 cm s^{-1} crosses the Equatorial Atlantic from east to west (or, in the Undercurrent and in the Countercurrents, from west to east) within less than 300 d. This shows that there is an appreciable inflow of water into the area by advection and that most of the water which enters the surface layer from below at a rate of 30 m yr^{-1} leaves the area at the eastern or western ends rather than through the poleward boundaries. The effect can of course be accounted for by assuming a higher upwelling rate. But this would almost certainly result in a discrepancy between the results of the two independent methods.

It seems, then, that we are still some way from reliable estimates of upwelling rates. The proposal of Broecker, Peng and Stuiver to use radiocarbon observations for an independent calculation is a very valuable one. Bomb radiocarbon concentrations will remain above natural background levels for another decade at least. It is certainly worth collecting more radiocarbon information to provide a good data base for an improved model of equatorial upwelling rates. □

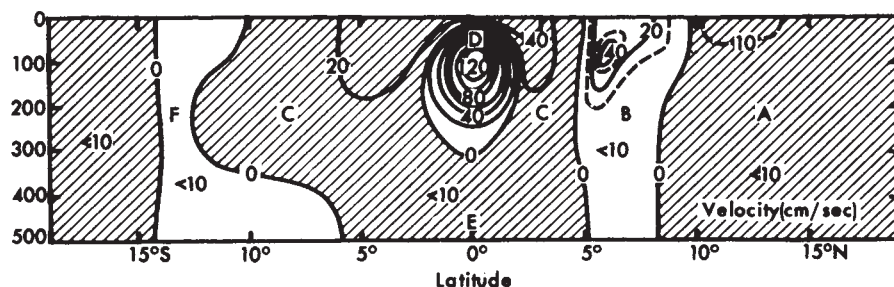


Fig. 2 A cross-section of the equatorial current system, showing the zonal component of velocity for the central Pacific Ocean. Westward flow is shaded, letters refer to individual components of the current system: A=North Equatorial Current, B=North Equatorial Countercurrent, C=South Equatorial Current, D=Equatorial Undercurrent, E=Intermediate Equatorial Current, F=South Equatorial Countercurrent. From Philander (*Rev. Geophys. Space Phys.* **11**, 513; 1973).

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A hundred years ago

Tides in the Bay of Fundy

HAVING resided for some years in the neighbourhood of this bay, I am able to give a little information respecting its tides. The bay splits into two at its inner end. One of these branches leads through a narrow channel into the broad basin

of Minas. The other, called Chegnecto Bay, is not interrupted by any such contraction, and is therefore more favourable for the formation of very high tides. This bay itself divides at its upper end into two, and one of these, called Chepody Bay, contracts very gradually for some thirty miles inland, forming the estuary of the Petitcodiac River. This is the place where the highest tides occur, and as far as I have been able to learn, their maximum height is 70 feet. A powerful "bore" is formed by the incoming waters. The captain of the steamer *Emperor*, which plied between St. John, N.B., and Windsor, N.S., informed me that the highest tide in any part of Minas Basin was about 55 feet.

A PETROLEUM spring, one boring of which has yielded 2,000 kilos in twenty-four hours, has been discovered at Pohar, in Austrian Poland.

ACCORDING to the report of H.M.'s Consul for Hiogo and Osaka, the Japanese claim that petroleum has been known in Japan for over 1,200 years, and it would certainly be curious if the numerous springs which exist in certain localities should have escaped notice in their immediate neighbourhood. It is doubtful, however, whether it was ever utilised, and certainly no attempt was made to refine it before the arrival of foreigners. From *Nature* **19**, 20 March, 458, 473; 1879.

articles

Neogene relative motion between the Pacific plate, the mantle, and the Earth's spin axis

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The northward rates of motion for the central equatorial Pacific during the past 24 Myr vary between ~ 20 – 30 mm yr^{-1} based on the migration of sedimentary facies, $\sim 30 \text{ mm yr}^{-1}$ based on the age and orientation of the Hawaiian ridge, and ~ 50 – 60 mm yr^{-1} based on palaeomagnetically determined sediment and basalt palaeolatitudes. To resolve the differences between hot spot and palaeomagnetic results we may need to invoke a long-term drift in the orientation of the geomagnetic field, or a northerly component of motion of the Hawaiian hot spot relative to the Earth's spin axis.

SIGNIFICANT differences between Neogene rates of motion of the Pacific plate relative to: (1) the Hawaiian hot spot, (2) the Earth's spin axis determined from palaeomagnetic data, and (3) the Earth's spin axis deduced from the distribution of equatorial sediment facies, suggest that one or more of the assumptions inherent in each method may be invalid.

Calculation of palaeolatitudes from palaeomagnetic data is usually based on the assumption that, when averaged for several thousand years, the Earth's magnetic field approximates that of a geocentric dipole coincident with the Earth's spin axis. This assumption is supported by palaeomagnetic results such as those of Opdyke and Henry¹, which are based on a worldwide distribution of sediment cores. Other studies conclude, however, that the time-averaged geomagnetic field is better represented by a dipole field slightly displaced into the Northern Hemisphere², or by a pair of unequal axial dipoles³. If either of the latter two results is valid, then palaeolatitudes determined assuming geocentricity will be systematically in error. At low latitudes, however, there is little difference between the geocentric and nongeocentric models in terms of the rate at which field inclination varies with latitude. Because most of the palaeomagnetic data used in this study are from latitudes of $< 10^\circ \text{ N, S}$, the choice of models has no significant effect on palaeomagnetically determined rates of plate motion, and, therefore, cannot be the source of discrepancy between palaeomagnetic results and either hot spot or facies migration results. It is not known whether the average pole positions of the geomagnetic field have remained coincident with the geographic poles over periods of time exceeding several tens of thousands of years. At present, however, there is no evidence of long-term net motion of the north geomagnetic pole towards the Pacific, which is the direction of movement that would be required to reconcile the present palaeomagnetic results with the less rapid northward rates of Pacific plate motion relative to the Hawaiian hot spot.

Other factors that may alter or obscure initial palaeomagnetic

remanence and thereby produce spurious palaeolatitudes, are magnetic overprinting, caused by chemical alteration in either rocks or sediments, and in non-indurated sediments, acquisition of systematic inclination error, caused by post-depositional grain rotation and compaction. Chemical alteration is usually recognised as systematic differences between the direction or magnitude of normal and reversed intervals within a magnetostratigraphic section, or by the behaviour of the remanent magnetism during alternating-field demagnetisation analysis. No such evidence of chemical alteration is observed in the present palaeomagnetic data, and Hammond *et al.*⁴ have shown that there is no discernible compaction-related rotation of palaeomagnetic vectors in equatorial Pacific pelagic radiolarian-rich clays as old as 30 Myr. Their results showed consistent inclination trends within individual chronologically overlapping cores recovered north and south of the Equator.

The present distribution of relatively thick equatorial sedimentary facies, originally deposited in equatorial regions of upwelling and high biological productivity, has been used^{5–8} as another means to determine the Cainozoic motion of the Pacific plate relative to the Earth's spin axis. Isopachs delineating progressively older sediments are systematically offset to the north. Poles and rates of rotation are determined by backtracking the locus of maximum sediment thickness, for a discrete time interval, to its original equatorial deposition site. It is difficult to quantitatively assess the reliability of plate motions based on the migration of equatorial sedimentary facies other than by their consistency with other independently derived results. The accumulation of sediment on a moving plate is governed by the interaction of dynamic factors including upwelling-induced surface productivity, the width of the area of upwelling, erosion, and CaCO_3 solution. Relatively brief hiatuses within a sedimentary section, due to nondeposition, dissolution, or erosion, for example, may be cumulatively significant as well as difficult to detect. Van Andel and Whaley⁹ have pointed out that maxima and minima in rates of sedimentation can vary in response to factors unrelated to the latitude of the deposition site. There is little doubt, however, that the distribution of equatorial sedimentary facies is strongly influenced by the motion of the Pacific plate.

According to the hot spot hypothesis of Wilson^{10–12} and Morgan¹³, linear volcanic chains are formed as the sea floor moves over magma sources below the lithosphere. Morgan¹³ originally defined 20 hot spots and postulated that they were surface manifestations of convection plumes that originate near the core-mantle boundary. He also suggested that hot spots might move relative to each other at maximum rates of about 5 mm yr^{-1} . Recent reconstructions (refs 14–17 and Morgan, personal communication) confirm that little or no relative motion is required between hot spots, suggesting that hot spots can be used as a global coordinate system to which plate motion

can be referred. Volcanic chains that were formed at hot spots can be used to determine the motion of plates relative to this coordinate system. Because the Hawaiian ridge–Emperor seamount chain is the most prominent and best studied hot spot trace on the Pacific plate, it provides the best estimate of the plate's motion with respect to a hot spot coordinate system. Epp's¹⁸ analysis used only this volcanic chain, and, therefore, made no *a priori* assumptions about the relative age of other volcanic chains on the Pacific plate. The poles Epp¹⁸ found, however, seem to be representative of other hot spot traces on the plate, suggesting that there has been little relative motion among Pacific hot spots for at least the past 25 Myr. In other studies, Clague and Jarrard⁷ used the Hawaiian, Cook–Austral, and Guadalupe chains to determine a pole position; their analysis was amended by Dalrymple *et al.*¹⁹ to incorporate a revised radiometric age for Midway. Minster *et al.*¹⁴ determined a pole position and rate of rotation from a systematic inversion of all available relative motion data and azimuths of hot spot traces. The poles and rates of rotation thus determined can be used to determine the northward motion of the Pacific plate relative to the hot spot coordinate system.

Palaeomagnetism of Pacific deep-sea sediments

The northward motion of the Pacific plate during the late Mesozoic and the Cainozoic seems to be responsible for the discrepancies, found for older sediments and volcanic rocks, between measured palaeomagnetic inclinations and those characteristic of the present latitude of the sampling site. Latitudinal migration of the Pacific plate has been inferred from results of paleomagnetic surveys of Pacific seamounts²⁰, palaeolatitudes from drilled basalts²¹, and palaeolatitudes from both drilled and cored sediments^{4,22}.

In the present study 27 chronologically overlapping piston cores, which collectively span the past 25 Myr, have been used to determine the northward motion of the central equatorial Pacific. The cores were recovered from the Central Pacific Basin (average depth 5.0–5.5 km), where extensive erosional surfaces make possible the recovery of relatively old sediments by piston coring²³. All of the cores contain homogeneous brown,

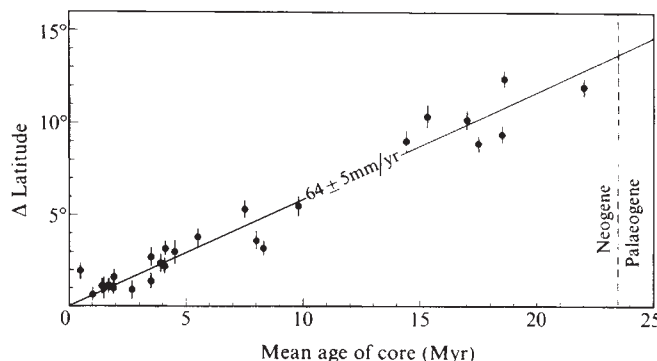


Fig. 1 Progressive increase, with increasing age, between the observed mean palaeolatitude and the coring site latitude (Δ latitude) for chronologically overlapping cores from the Central Pacific Basin. Vertical bars represent the standard error for the mean palaeolatitude of each core. Age span and Δ latitude value for the cores are shown in Table 1.

radiolarian-rich clays (Table 1). Average core length is about 13 m and average sediment accumulation rate is about 3 mm per 10^3 yr. This relatively low accumulation rate means that each sample from the cores contains, on the average, a depositional record spanning about 6,000 yr. Magnetic secular variation is therefore largely averaged out within single samples.²⁴ The cores were dated by correlating their radiolarian and magnetostratigraphy to the time scale of Thayer and Hammond^{25,26}. The K76 core series (Table 1; Fig. 1) is fully orientated, which allows an unambiguous determination of both polarity and the hemisphere in which the cores were deposited²⁷.

The stability of depositional magnetic remanence was established by standard alternating-field partial demagnetisation analysis of at least six samples from each core^{4,28}. After the stable remanence was resolved, individual inclination values in each core were converted to palaeolatitudes by use of the geocentric dipole formula. A mean palaeolatitude was calculated for each core by first determining the mean palaeolatitude for each polarity interval, and then dividing the sum of the absolute values of the polarity interval means by the total number of polarity intervals.

The mean palaeolatitude for each core was normalised for its core site latitude⁴ and then plotted against the mean age of the core (Fig. 1). Each core was sampled at 10-cm intervals, so the average northward rate indicated in Fig. 1 is based on nearly 3,500 discrete palaeolatitude determinations. A linear regression of the differences between mean palaeolatitude and coring site latitude (Δ latitude) versus mean age, assuming mean core age to be the independent variable, indicates an average rate of palaeolatitude change for the central Pacific of about 64 mm yr⁻¹ throughout the Neogene. This rate agrees with that determined in a similar manner by Hammond *et al.*⁴ (using seven cores) for the interval 0 to –13 Myr, but is considerably slower than their previously determined rate of about 110 mm yr⁻¹ for –13 to –21 Myr. The distribution and resolution of the present data preclude a reliable determination of variations in the rate of northward motion during the Neogene, although such variations may have occurred as a result of reorganisation of plate boundaries and change in the rate and/or direction of seafloor spreading. At present, palaeomagnetic data are insufficient for reliable estimation of an average northward rate of plate motion during the Palaeogene, but the Neogene rate shown in Fig. 1 is compatible with approximately 30° northward displacement of the Pacific plate since the Cretaceous^{5,20}.

Comparison of results

Since northward rates of motion for different locations on a plate vary with distance and azimuth to the pole of rotation, DSDP Site 166 (3.76° N, 174.08° W), located in the Central Pacific

Table 1 Cores taken from the Central Pacific Basin

Core no.	Coring site	Water depth (m)	Recovery (m)	Age span of core (Myr)*	Δ latitude (deg)
S68-24	2.0° N, 178.8° W	5291	21.5	16.7–27.0	12.0
M70-10	1.6° N, 166.3° W	5200	9.3	16.4–17.5	10.2
M70-14	0.7° N, 162.5° W	5200	11.3	0.1–0.8	1.9
M70-16	3.2° S, 160.3° W	5505	11.2	0.3–3.6	1.6
M70-17	7.5° S, 161.5° W	4730	11.5	3.7–12.4	3.6
M70-38	2.3° S, 171.0° W	5360	15.8	16.4–21	12.4
M70-39	2.5° S, 173.3° W	5412	21.8	2.3–4.8	2.7
M70-76	3.0° N, 174.9° E	5015	12.9	12.5–16.4	9.1
K72-36	2.4° S, 177.8° W	5545	9.8	16–19	8.9
K72-37	2.1° S, 178.9° W	5310	12.2	3.9–11.0	5.3
K72-38	5.4° N, 164.4° W	4658	12.1	0.0–2.9	1.0
K72-46	2.3° N, 164.2° W	5235	12.3	0.6–4.7	0.9
K72-48	4.2° N, 165.8° W	5477	12.0	1.6–5.3	1.4
K76-03	7.6° S, 169.7° E	4745	8.1	0.0–2.7	1.1
K76-04	7.1° S, 171.0° E	5000	15.8	0.4–3.4	1.1
K76-06	4.7° S, 173.4° E	4735	11.0	14–16	10.4
K76-07	4.4° S, 174.1° E	5170	11.7	3.6–4.6	2.2
K76-08	2.8° S, 177.9° E	5290	11.7	3.0–4.7	2.3
K76-09	2.8° S, 177.9° E	5295	10.6	3.0–6.0	3.0
K76-10	1.1° S, 179.5° E	5355	11.8	1.3–4.8	3.2
K76-12	0.9° N, 178.6° W	5195	11.7	3.0–11.0	3.8
K76-14	2.7° N, 178.7° W	5390	11.1	0.2–2.1	0.6
K76-15	2.9° N, 178.5° W	5260	11.3	5.3–11.2	3.2
K76-16	2.9° N, 178.5° W	5380	17.9	0.4–3.3	1.0
K76-17	4.6° N, 176.5° W	5170	17.0	13–24	9.4
K76-18	4.6° N, 178.6° W	5240	16.9	8.0–11.5	5.5

*Ages greater than 5 Myr are based on correlations of each core's radiolarian and magnetostratigraphy to the Neogene sediment-based reversal time scale of Thayer and Hammond^{25,26}. For this interval, a conservative estimate of the accuracy of a given age is about ± 0.5 Myr (ref. 33).

Table 2 Pole positions and rate of rotation for various age ranges determined by different authors

Author	Pole		Age range (Myr)	Rate of rotation (deg Myr ⁻¹)
Epp	65° N	40° W	0–23	0.86
	59° N	54° W	23–42	0.67
Minster <i>et al.</i>	67.3° N	59.4° W	0–10	0.83
Clague and Jarrard and Dalrymple <i>et al.</i> van Andel	69° N	68° W	0–42	0.83
	67° N	59° W	0–25	0.83
	67° N	59° W	25–50	0.25
Lancelot and Larson	67° N	45° W	0–40	0.5

Basin (and central to the location of the palaeomagnetically studied sediment cores), was chosen as a convenient location where palaeomagnetically-derived northward rates of plate motion could be compared with the northward rates of motion determined from migration of sediment facies and from hot spot traces. Using the pole positions and rates of rotation determined by the investigators listed in Table 2, Site 166 was rotated from its present location to its position at –24 Myr. In Fig. 2 the change in latitude (Δ latitude) of Site 166 determined by these rotations is compared with the palaeomagnetically determined change in latitude. The palaeomagnetic data were fit by a straight line which indicates a northward motion of 64 mm yr⁻¹. The other rotations show a slight increase in the northward rate of motion with age which results from the change in position of DSDP Site 166 relative to the poles of rotation. The northward rates determined from the hot spot analyses of Epp¹⁸, Clague and Jarrard⁷ together with Dalrymple *et al.*¹⁹, and the plate inversion analysis of Minster *et al.*¹⁴ are similar and about 30 mm yr⁻¹. The consistency of these analyses supports the result.

The sediment facies analysis by van Andel⁶ requires a similar northward motion, 33 mm yr⁻¹, at Site 166. The more recent equatorial facies analysis of Lancelot and Larson⁸, on the other hand, indicates a considerably slower northward rate, about 20 mm yr⁻¹, for the same time interval and location. Winterer's⁵ sediment facies analysis (not shown) yields a northward velocity of about 43 mm yr⁻¹.

There are relatively few other Neogene palaeomagnetic results from the Pacific plate which can be compared with the foregoing results. Grommé and Vine²⁹ determined an average palaeolatitude from samples of 13 basalt flows recovered in a

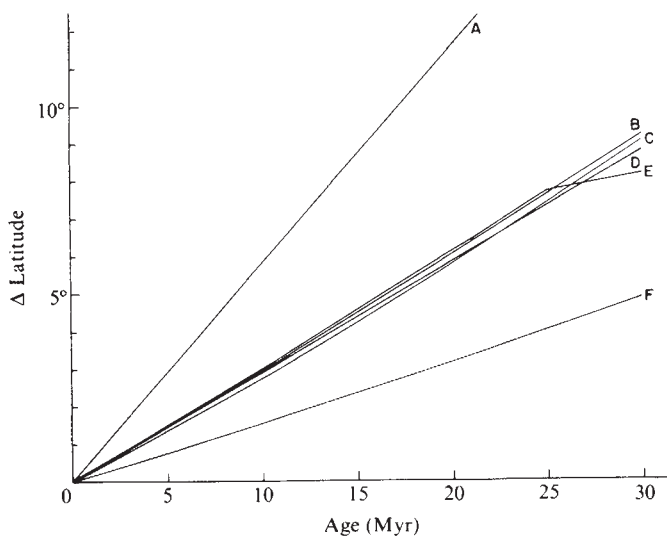


Fig. 2 Change in latitude versus age for DSDP Site 166 from: A, sediment palaeomagnetism; B, Minster *et al.*¹⁴; C, Epp¹⁸; D, Clague and Jarrard⁷ with a rate of rotation determined by Dalrymple *et al.*¹⁹; E, van Andel⁶; F, Lancelot and Larson⁸.

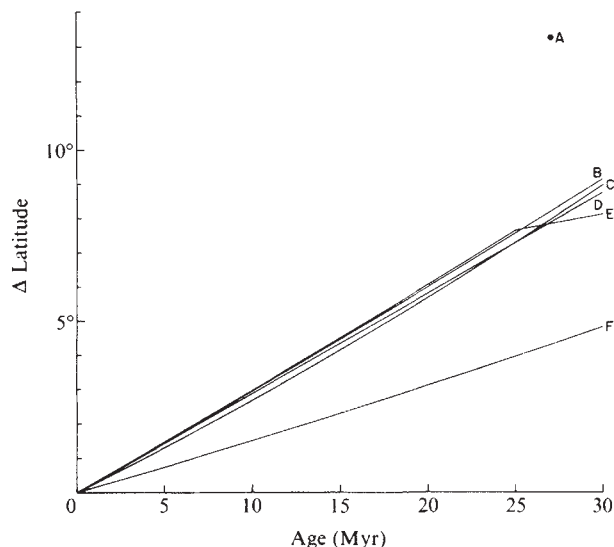


Fig. 3 Change in latitude versus age for Midway. Midway data from Grommé and Vine²⁹ and Dalrymple *et al.*¹⁹ Other lines from the same sources as in Fig. 2.

deep drill core from Midway Island (28.2° N, 177.6° W). Thus Midway is another location where the Neogene northward motion of the Pacific plate determined by the above analyses can be compared (Fig. 3). The northward velocities at this location differ only slightly from the Site 166 results, and the discrepancies between the various analyses are just as pronounced. The palaeomagnetic data represented by point A in Fig. 3 indicate that Midway Island has been displaced northward by ~13° during the past 27 Myr. Using the average rate of northward displacement of Midway during this interval of time (52 mm yr⁻¹) to constrain the Hawaiian ridge model of Epp¹⁸, an average northward velocity, for the Central Pacific Basin, of 55 mm yr⁻¹ is obtained. Thus the Midway palaeomagnetic rate is consistent with the Central Pacific Basin palaeomagnetic results in that it also is significantly more rapid than the other rates shown in Fig. 2.

Results of a recent study by Marshall³⁰, of mid-Cretaceous to mid-Miocene basalts recovered at several north Pacific DSDP sites, are consistent with the Midway and the Central Pacific Basin palaeomagnetic results inasmuch as they, too, indicate a higher rate of northward plate motion than do hot spot and sediment facies studies.

Discussion

The results in Figs 2 and 3 show significant and consistent discrepancies among independently derived Neogene rates of northward motion of the Pacific plate. Palaeomagnetically derived rates are roughly twice as fast as those determined by backtracking equatorial sedimentary facies to their original deposition sites.

The sediment facies analyses describe the motion of the plate relative to the spin axis, and therefore should be consistent with the palaeomagnetic analyses. As shown in Figs 2 and 3, however, the predicted rates of northward motion based on sediment migration are considerably slower than those based on palaeomagnetism of both sediments and basalt. The inherent difficulties in sediment facies analyses discussed above may be responsible for the differences among the van Andel⁶, Lancelot and Larson⁸ and Winterer's⁵ results as well as the difference between the sediment facies results and the palaeomagnetic results. If palaeomagnetically determined rates do provide an accurate indication of plate motion relative to the spin axis, the agreement between facies results and those based on hot spots may be fortuitous.

The palaeomagnetically derived rate of about 64 mm yr⁻¹ appears reliable from the standpoint of the consistency and the

large amount of data from which it was determined. Likewise, the agreement between the results of Epp¹⁸, Minster *et al.*¹⁴, and the combined work of Clague and Jarrard⁷ and Dalrymple *et al.*¹⁹ strongly indicates that 30 mm yr⁻¹ is a reliable rate for the northward motion of the Pacific plate relative to the hot spot coordinate system. If the Earth's magnetic field is fixed with respect to the spin axis and the palaeomagnetic results indicate the true velocity of the Pacific plate with respect to the spin axis, then, to resolve the difference between the palaeomagnetic and hot spot rates, the Hawaiian hot spot must have moved northward relative to the spin axis at an average rate of about 30 mm yr⁻¹. The implications of this motion for the motion of the mantle with respect to the spin axis depends on the origin of hot spots. If hot spots are fixed with respect to the asthenosphere, then any hot spot versus spin axis motion requires only that the asthenosphere move relative to the spin axis. If, on the other hand, hot spots are the surface manifestations of plumes that ascend from the core-mantle boundary³² then the entire mantle must be moving relative to the spin axis. As the total vector of motion of the mantle is unknown, the absolute mantle velocity cannot be determined; however, if this motion is the result of some mantle-wide convection system, there should be southerly motion at some other location. Burke *et al.*¹⁷ found that hot spots in the North Atlantic have moved northward relative to the spin axis, and hot spots in the South Atlantic have moved southward. To conserve matter, the mantle under Asia must be moving southward relative to the spin axis; however, the southward motion of South Atlantic hot spots suggests a more

complicated convection pattern. An intermediate possibility is that different plumes come from different depths in the mantle. This conceivably could allow a complex mantle convection system while retaining the fixed nature of hot spots.

An alternative explanation for the differences between plate motion rates, especially in view of the close agreement between van Andel's⁶ results and the hot spot results, is that the palaeomagnetic results are systematically biased with increasing age. As compaction-related inclination error and tectonic tilting of the Central Pacific Basin can be ruled out, the possibility that the north geomagnetic pole was moving towards the Pacific at a rate of approximately 30 mm yr⁻¹ during the Neogene must be considered. Andrews³² analysis of polar wander during the Mesozoic and Cainozoic indicates that the field was moving relative to the Pacific plate during the Neogene, but the apparent motion was in essentially the opposite direction needed to reconcile the palaeomagnetic, sedimentary facies, and hot spot results. If Andrews³² conclusion is correct, the palaeomagnetic rates, relative to the Hawaiian hot spot, are even more rapid than indicated in Figs 2 and 3.

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Tidally generated internal wave packets in Massachusetts Bay

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Observations in Massachusetts Bay of high-frequency internal wave packets indicate that they are caused by lee waves generated outside a submarine bank at the Bay's seaward margin during ebb tide. The lee waves propagate into the Bay as the tide turns to flood, steepen nonlinearly, and develop into the packet. A 200-kHz acoustic back-scattering system detected the evolution of the packets. Large overturning events were observed acoustically and in density profiles. Plankton distributions undergo strong vertical displacements and mixing associated with the wave packet passage.

HIGH-FREQUENCY internal wave packets in Massachusetts Bay^{1,2} (Fig. 1) have been studied to understand their generation and propagation, to describe the biological variability in the plankton caused by their passage, and to further evaluate the use of high-frequency acoustic backscattering systems as remote sensors of fluid processes in the ocean. Previous work on the physical properties of internal wave packets generated by tidal flows over topography¹⁻⁶, by atmospheric forcing of internal surges^{7,8}, or from unknown causes⁹⁻¹¹, has concentrated on packet propagation. Fewer generalisations seem possible about packet generation because it is more dependent on the particular forcing field and the local topography. D. Farmer and J. Smith (personal communication) and also T. Maxworthy¹², have developed conceptual models for the topographical generation

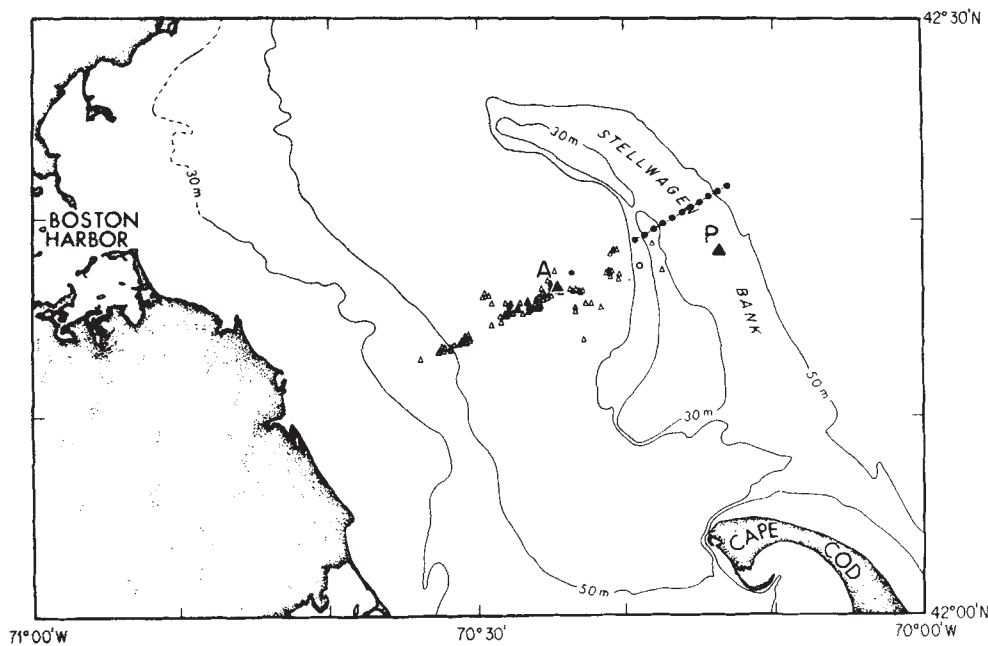


Fig. 1 Experimental area in Massachusetts Bay. A and P mark mooring sites. ●, Locations of Sippican T-11 expendable bathythermograph profiles. △, Positions at which internal wave packets were acoustically observed. *, Position at which acoustic record of Fig. 4a was obtained. Site P was a subsurface mooring (float at 22 m; water depth nominally 35 m) with a General Oceanics 2011 film-recording current meter at 25 m depth. Site A was a subsurface mooring (float at 22 m; water depth nominally 80 m) with an Aanderaa RCM4 current meter at 25 m depth, and a surface mooring carrying an Aanderaa TR1 thermistor chain with sensors at 3-m intervals between 5 and 32 m. ○, The deployment location of VCM2 (Fig. 3); VCM3 (Fig. 3) was very near site A.

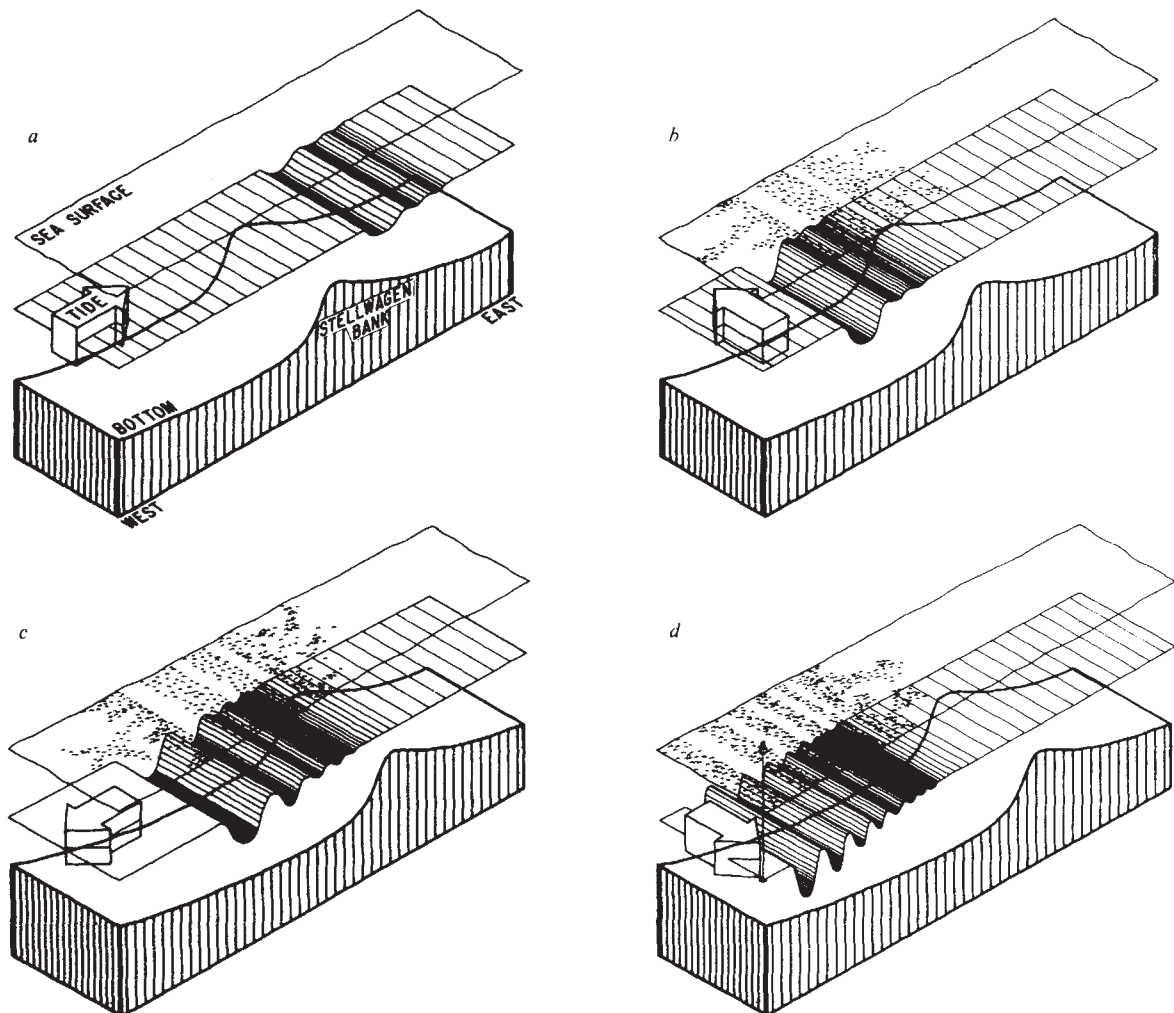


Fig. 2 Schematic diagram of internal wave packet generation as a lee wave during ebb tide over Stellwagen Bank (a), followed by nonlinear steepening after the tide turns (b). The turn of the tide allows the packet to propagate over the bank towards shore. Undulations then develop behind the steep front (c and d). See Fig. 4a for 200-kHz acoustic record of a developed packet.

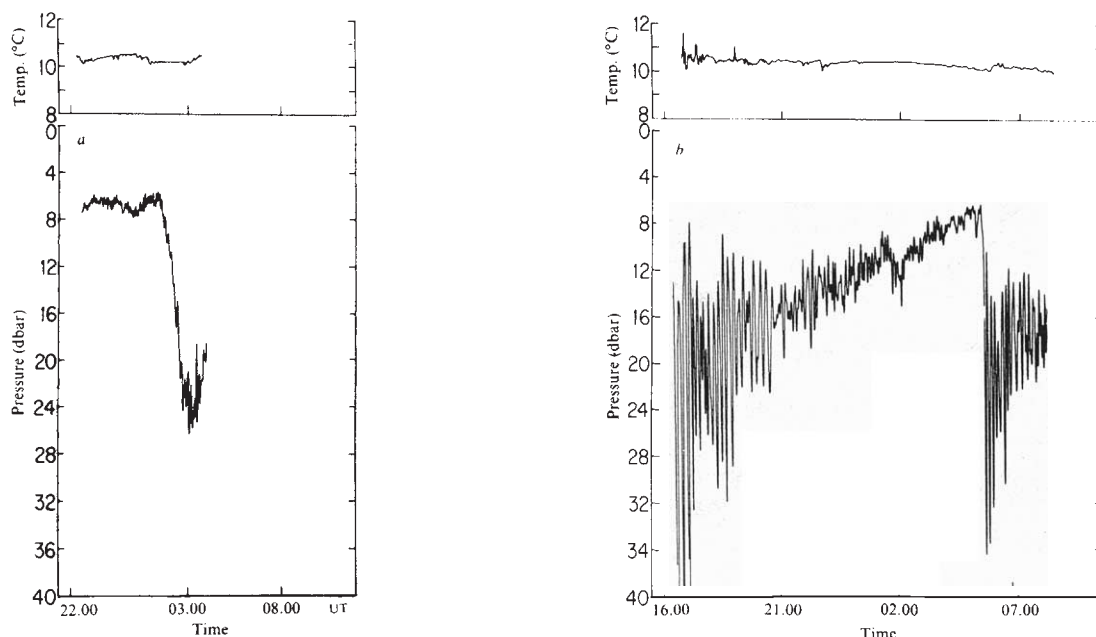


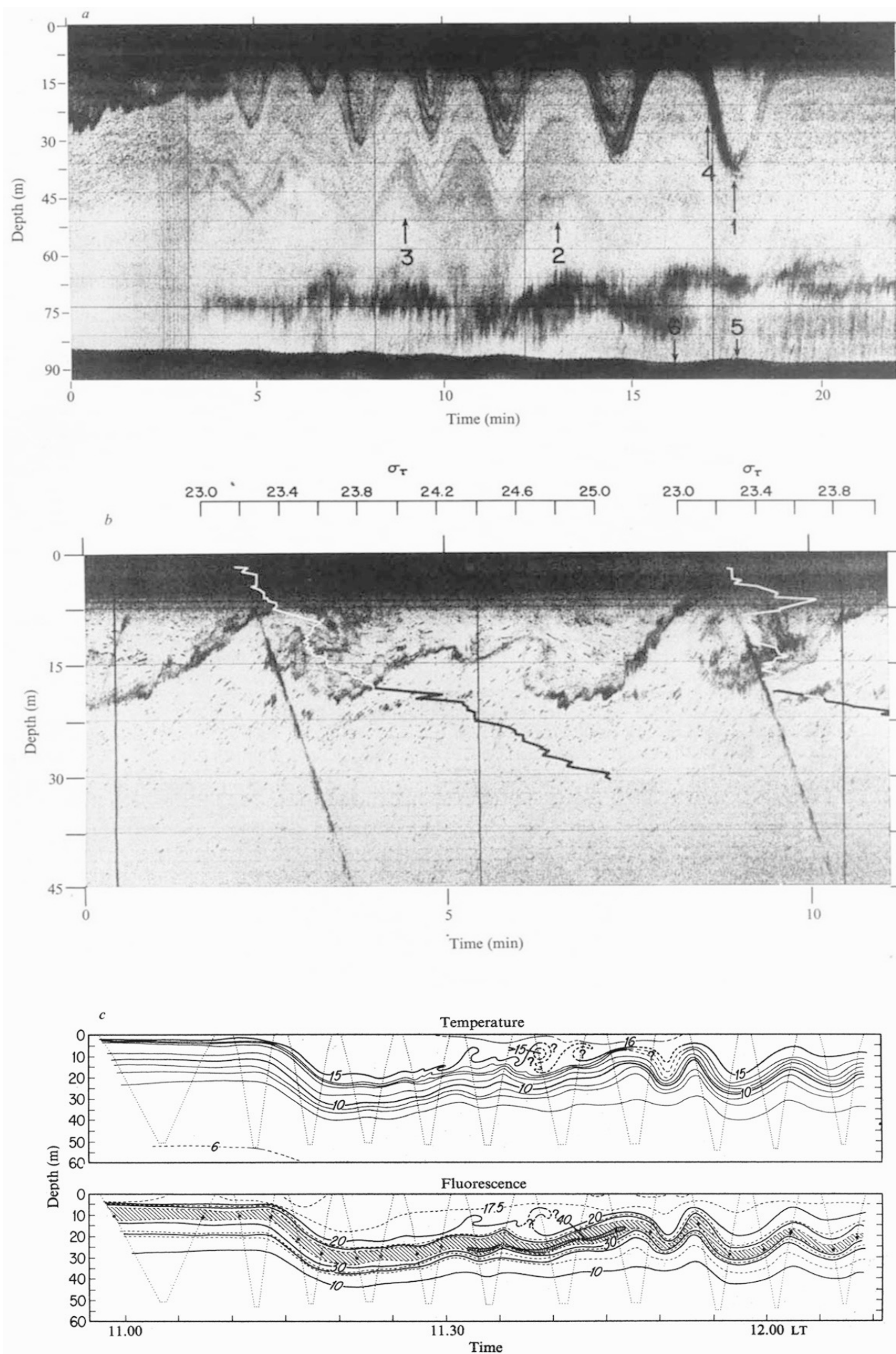
Fig. 3 Temperature and depth records from a modern version of the Swallow float vertical current meter (VCM)^{17,18} at positions shown in Fig. 1 *a*, VCM2 on 31 August; *b*, VCM3 1 September. VCM2 drifted about 0.6 km WSW and VCM3 about 2.3 km SSW during their respective deployments. The VCM3 trace shows the two internal wave packets that followed the single packet of the VCM2 trace. The VCM internally records temperature, pressure and rotation (vanes on the instrument rotate it about a vertical axis when there is vertical flow relative to the instrument). An acoustic signal from the VCM allows shipboard tracking while the instrument is deployed. Because the temperature is nearly constant at 10.2 °C (sensor time response <1 min), and because the rotation showed insignificant vertical currents past the VCM (less than 20 cm vertical water displacement), the VCM depth accurately portrays the fluctuating depth of the 10.2 °C isotherm. The record of isotherm depths from site A-moored thermistor chain during the VCM3 packet and results of CTD vertical profiling both substantiate this interpretation.

of internal wave packets by a time-varying tidal flow which are good descriptions of our observations in Massachusetts Bay. Specifically, the ebb tide flow over Stellwagen Bank (Fig. 2) generates a lee wave on the eastern side. When the tide turns to flood, the lee wave propagates into Massachusetts Bay (heading about 240 °T), steepens due to nonlinear effects¹³, and develops undulations due to an imbalance of dispersion and nonlinear effects¹⁴. In analogy to other locations^{3,4}, we would expect to observe wave packets outside Stellwagen Bank due to lee waves inside during flood tide. As most of our observations were inside the Bank, we did not see outgoing waves. They may not be present because of the asymmetry of the Bank and tidal flow.

Lee wave propagation

The occurrence of lee waves on the eastern side of Stellwagen Bank depends on an ebb tide flow with a subcritical internal Froude number¹⁵. Our measurements, together with those of ref. 16, showed the flow to be subcritical using either a two-layer fluid or a continuous-gradient model. The presence of the lee waves was substantiated by a transect of 11 bathythermographs across the Bank during ebb tide (Fig. 1). The 10 °C isotherm (in the thermocline) descended from 8 m over the Bank to 20 m at about 7 km to the ENE with oscillations that may be the expected lee waves.

Fig. 4 *a*, 200-kHz acoustic record of an internal wave packet observed at the position marked * in Fig. 1. The period of the waves are Doppler shifted by the ship's speed of about 2.5 knots (128 cm s^{-1}) as it travelled from the rear to the front of the packet parallel to the packet's propagation direction (240°; left to right in figure). The seasonal thermocline is displaced by 30 m (arrow 1) and the stratified point scatterers (zooplankton?) at 30 to 40 m are displaced more than 20 m (arrows 2 and 3). The heavy acoustic backscattering in the vicinity of arrow 4 and extending in an oscillatory pattern throughout the figure is possibly caused by turbulent mixing in the thermocline. The asymmetry of the wave field, with narrow troughs and broad crests, is determined by the depth of the mixed layer²⁰⁻²¹. Similar asymmetrical waves have been observed acoustically in the lower atmosphere²². The heavy scattering near 75 m is probably caused by euphausiid and mysid shrimp. The oscillations in bottom depth (arrows 5 and 6) are due to variations in the travel time of the sound pulses resulting from the thermal structure of the waves; that is, the water column above arrow 6 is mostly cold (slower speed of sound). *b*, The 200-kHz acoustic record of overturning instabilities associated with the internal wave packet (propagating from right to left) at site A (Fig. 1) between 11.30 and 11.41 LT, 30 August 1977. This portion shows two overturning events; the direction of overturning (left to right) is opposite that of packet propagation. Superimposed are the corroborating density profiles obtained by repeated vertical casts of the CTD. The instrument's path is seen as the oblique traces in the acoustic record. The ship was drifting during the observations. The winds during these observations (and for most of the experiment) were $<2 \text{ m s}^{-1}$. *c*, Profiles of temperature and chlorophyll fluorescence for the same wave packet as in Fig. 4*b*, but between 10.58 and 12.10. The vertical exaggeration is about twice that of the acoustic record; isotherms and 'isofluors' are drawn between profile data points using the acoustic imagery as a guide. The wide hatching depicts fluorescence values greater than 37.5 arbitrary units, the narrow hatching greater than 40.0 units. ●, Within the hatched areas are at the depth of the chlorophyll maximum. The light level at the chlorophyll maximum layer before the packet arrival was 18% of ambient (range 14–22%) [Secchi Disk depth: 11 m, extinction coefficient $k = 0.17$]. Immediately after arrival, the mean level was 3% (range 1–13%). Four hours later, the mean light level was still only 3.5%. During the time shown the ship was drifting with the local currents such that the observations (11.15 to 11.30 LT) are along the first trough of the wave packet, then slowly scanning back (after 11.30) through the packet as the current turned from west to SSW.



Although we believe that only lee waves were formed over Stellwagen Bank during our observations, certain conditions (sufficiently high Froude number) could generate a turbulent internal hydraulic jump. This has been observed in fjords with blocking sills³. The potential thus exists at Stellwagen Bank for strong biological effects due to the mixing. In its absence, however, the propagation of the lee wave into Massachusetts Bay seems to be a wave phenomenon with no significant net transport of water or plankton by the waves alone.

Two sets of data (Fig. 3) support the picture of wave propagation shown in Fig. 2b and c. The 10.2°C isotherm at the front of a newly developed wave packet (VCM2) initially deepens at about 18 cm s⁻¹, with few oscillations occurring during the next hour. At a later stage of wave development (VCM3), the isotherm deepens at 38 cm s⁻¹ with strong following oscillations. Numerous 200-kHz acoustic records¹⁹ obtained between Stellwagen Bank and site A also support the model. Neither of the VCM floats was transported by the wave packets; this supports the conclusions of the preceding paragraph.

The propagation of internal wave packets has been described by a form of the Korteweg-de Vries equation^{2-4,7}. Possible solutions include a modulated, undulatory wave packet in which the modulation has the form of solitary waves. The record of isotherm depth from VCM3 (Fig. 3) fits this solution; the 8–10-min period oscillations have a modulation envelope of about 90 min period; about three envelope oscillations are distinguishable. Long period modulations are also present in the thermistor chain record from site A.

Wave packets were repeatedly observed with the acoustic system (see Fig. 4a) as they propagated through Massachusetts Bay. Some of these acoustic records show large shear instabilities (Fig. 4b). These are similar to turbulent shear flow in the atmosphere²³ and laboratory²⁴⁻²⁶. Densities calculated from vertical profiling conductivity-temperature-depth (CTD)²⁷ casts through two events (Fig. 4b) show strong inversions. This supports the interpretation of these structures as overturning instabilities. The direction of overturn, being opposite to the wave propagation direction (Fig. 4b), argues for a shear opposed to the wave propagation²⁶, that is, the surface velocities are

against the waves, the deep velocities are with them. We have no direct measurements of shear, but note that $2 \times 10^{-2} \text{ s}^{-1}$ levels have been observed at the site¹⁶.

Redistribution of chlorophyll and zooplankton

Figure 4c shows the overturning events reflected as inversions in the vertical profiles of temperature and chlorophyll fluorescence. These events redistribute the chlorophyll and zooplankton. As no nutrient data were collected, we do not know if the surface layer is enriched with nutrients mixed from below. Because the geographical regions within which internal waves break are restricted both spatially and temporally, the vertical effects of mixing would be reflected in heterogeneous horizontal distributions of chlorophyll, zooplankton, and, if nutrients are affected, primary production.

Phytoplankton production may be affected by the interaction of the diel light cycle with the chlorophyll depth variations caused by internal wave packets acting over the semi-diurnal tidal cycle of 12.4 h. A mathematical model of internal wave effects on a chlorophyll maximum layer at the depth of the 10°C isotherm showed the maximum daily peak illumination level (12 h day length) experienced during a 2-week tidal cycle varies by a factor of 2.3, whereas the minimum total daily integrated illumination is 67% of the maximum. In these conditions, production is affected both by simple light limitation over the 2-week tidal cycle²⁸ and by the more complex physiological effects of rapid light variation during any one day²⁹.

Zooplankton distributions were sampled at site A using a Longhurst-Hardy plankton recorder (LHPR)³⁰. These data and the simultaneous acoustic records showed no evidence for any behavioural response rapid enough to suggest that the plankters are other than passive markers of the wave field. Plankton distributions taken by the horizontal LHPR tows, however, do vary markedly with the passage of internal waves. In Fig. 5, the abrupt change in the temperature and salinity signatures of the internal waves at sample 50 are mirrored by chlorophyll fluorescence and appendicularians, but not by amphipods and

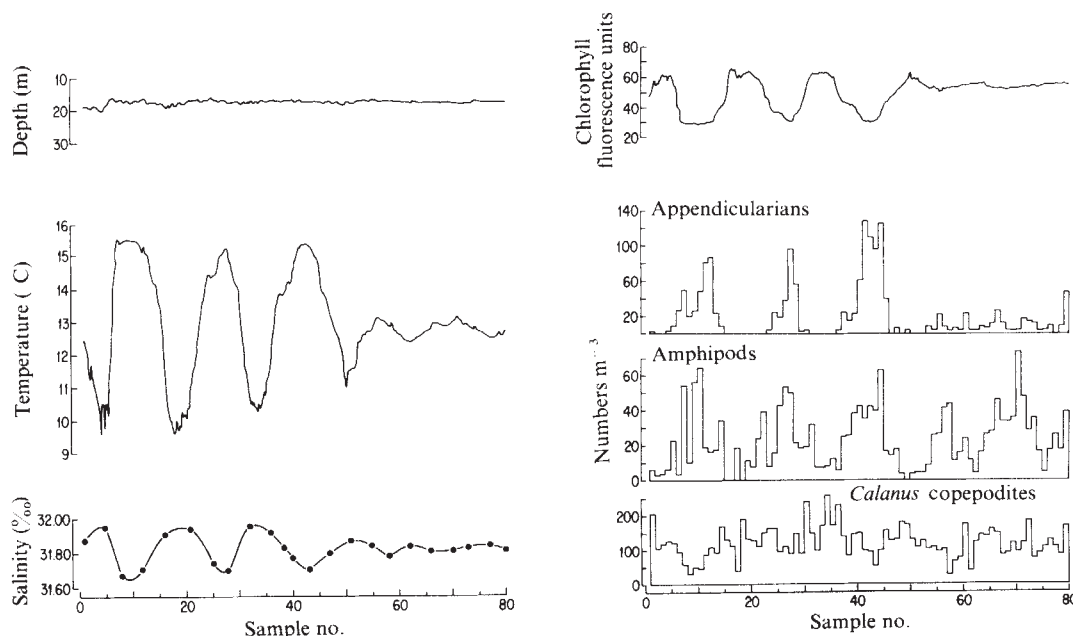


Fig. 5 Data from the Longhurst-Hardy plankton recorder tow taken at constant depth (17.5 m) through the first part of the internal wave packet at site A between 09.31 and 09.52 LT 28 August 1977. The record begins just after the leading edge (first trough) of the packet passed the ship. The direction of the tow was into the wave packet propagation; the tow speed was about 75 cm s⁻¹; tow length 980 m; sample length 12.2 m; mean volume filtered per sample 0.25 m³ (no net was used preceding the recorder box). The depth of the tow, temperature, salinity and chlorophyll fluorescence records are from the CTD and *in situ* fluorometer mounted on the plankton recorder vehicle. Other tows (oblique), taken to determine vertical distributions, showed the appendicularians and amphipods to be restricted to the warmer surface waters, whereas the *Calanus* copepodites occurred in greatest abundance in deeper water of temperature less than about 12–14 °C.

Calanus copepodites. Another tow taken at 1.5 m through the first three crests following the leading edge of an internal wave packet showed abundant *Calanus* copepodites at the surface in the third crest; water of almost the same temperature, but with few copepodites present, occurred at the surface in the preceding two crests. The 200-kHz acoustic records accompanying all the tows provided a valuable picture of the vertical distribution throughout the water column of scattering from both organisms (that is, point scattering) and from possible temperature and velocity fine structure.

Isotherm following has been suggested as a way of removing the confounding effects of internal waves on studies of plankton pattern³¹. The rapid rate of isotherm depth variation (see Fig. 3) makes such a task very difficult. Our observations show that variability of periods similar to those of the internal waves is effectively removed, but there is increased short period variability due to unavoidable excursions above or below the target isotherm depth and the impossibility of following isotherms in regions of overturning. Long period (greater than the internal wave period) variations in horizontal abundance, however, are clearly evident; these would not be easily detected by towing at constant depth.

Conclusions

The generation of the wave packets can be explained by the models of Maxworthy and Farmer (Farmer's internal hydraulic jump is more violent than our observations) and the steepening and propagation are consistent with earlier studies. We see suggestions of envelope behaviour in the modulation of the wave packets, and our acoustic observations show large overturning instabilities substantiated by density inversions in the CTD profiles. Strong inhomogeneities in the vertical and horizontal plankton distributions are caused by the passage of the internal waves; the waves also have potential effects on primary production in the Bay. Acoustic records (200 kHz) are particularly valuable in providing a real-time kinematic view of

the scattering field due to the combination of plankton and internal waves.

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Pliocene footprints in the Laetolil Beds at Laetoli, northern Tanzania

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Recent excavation of the tuffs of the Laetolil Beds in Tanzania has revealed the presence of a large variety of footprints from the Pliocene. Many of these prints can be correlated with fossilised remains of Pliocene animals found in the same area.

IT was stated previously¹ that the name Laetolil would be used in preference to either Garusi or Vogel River for the area where the Laetolil Beds are exposed. Laetolil, as stated then, is an anglicisation of the Masai word Laetoli and was first used by Kent². The Tanzanian authorities have now asked that the term Laetolil should be dropped in favour of Laetoli. The name Laetoli will be used for the area, but the Pliocene deposits will continue to be known as the Laetolil Beds, as established in 1976¹.

The Laetolil Beds (Figs 1, 2) are dominantly tuffs which have a maximum known thickness of 130 m and are divisible into upper

and lower units. Nearly all the fossils have come from the upper unit which is 45–60 m thick.

In 1975 three marker tuffs had been identified in the upper unit of the Laetolil Beds¹. Since then, more than a dozen widespread air-fall tuffs (Fig. 3) have been recognised, permitting detailed correlations.

Description of the footprints tuff

The footprints tuff is divisible into two units of differing lithology and structure. The lower unit, 7–8.6 cm thick, is relatively uniform in thickness and is characterised by widespread ash layers of even thickness, commonly with rainprinted surfaces. The upper unit, generally 5–7 cm thick, thins over the higher areas of the lower unit and thickens in depressions to eliminate the undulations preserved by the mantle bedding of the lower unit.

Several unusual features of these tuffs can be explained by composite ash falls of natrocarbonatite ash and melilitite lava globules. The ash must have been cemented rapidly to have

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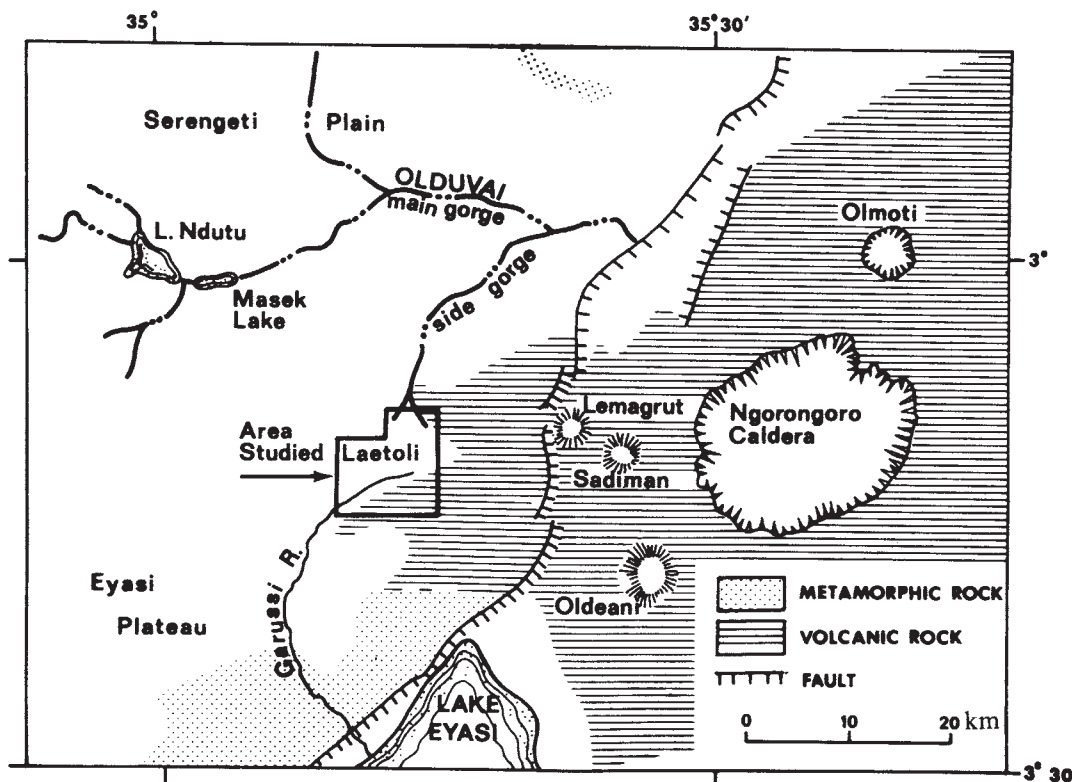


Fig. 1 Map of the southern Serengeti and volcanic highlands showing the position of the Laetoli area.

prevented erosion of the sand-sized lava globules by wind in this semi-arid climate in which 80–85% of the sediment was wind-worked. Natrocarbonatite ash would have dissolved incongruently in rainfall to yield soluble carbonates, which would have crystallised under the heat of the Sun to cement the ash layer in a few hours.

Footprints were made on at least six different surfaces but are by far the most common at two levels (Fig. 4). Prints of birds and

hares are common to all levels, but prints more than about 10 cm in diameter have been found only in the upper unit. Particularly striking is the number of elephant prints in the higher levels compared to their apparent absence below.

On the basis of the available evidence a tentative history of the footprint tuff begins near the end of the dry season and continues into the rainy season. The first showers of ash fell on a relatively bare, nearly flat landscape with scattered *Acacia* trees. The ash layers which constitute the lower unit were cemented by intermittent showers near the beginning of the rainy season. A few times between showers extruded eolian sediments, together with some of the air-fall ash, were redeposited by wind. The sharp contact at the base of the upper unit may mark the onset of the rainy season. Stratification in the upper unit is compatible with sheetwash produced by heavy showers, but is unlike that of the lower unit in which individual ash layers vary little in thickness over a distance of 5 km. The smaller amount of calcite in the upper unit may have resulted from either heavy rains or a smaller original content of carbonatite ash, or both. Thus, the abrupt appearance of footprints of elephants and other large animals in the upper unit may represent, at least in part, their migration which accompanies the rainy season.

The 1975 and 1976 field seasons at Laetoli were devoted to study of the geology of the area and the collection and excavation of fossil vertebrates and molluscs from the Laetoli Beds and later deposits. The age of the upper, fossiliferous part of the Laetoli Beds was established at 3.6–3.75 Myr (ref. 1).

While visiting the Laetoli camp during 1976 Dr Andrew Hill observed a number of depressions in the surface of a fine-grained tuff exposed in a river bed. These proved to be footprints of birds and mammals ranging from elephant and rhinoceros to carnivores and hares, which had been exposed by natural erosion and weathering.

The first site where footprints were observed (site A in Fig. 2) lies just south of the Garusi River in fossil Locality 6. An area of ~490 m² has been exposed by natural erosion and by excavation. To the south-west, at a second site (C) there are ~156 m² of the footprint-bearing tuff exposed. Both these localities were studied in detail in 1977. Five further areas where the footprint-bearing tuff is well exposed are also known but have not yet been completely studied.

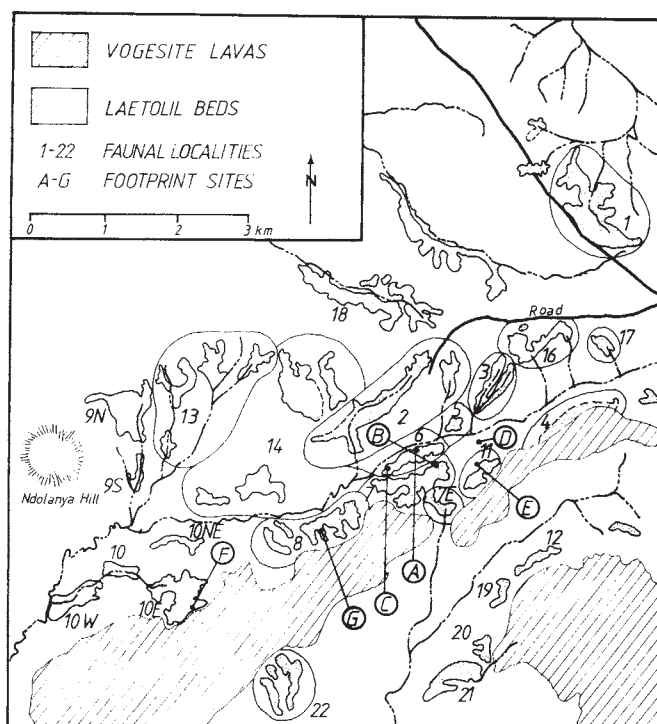


Fig. 2 Map of the fossil localities and footprint sites at Laetoli.

Table 1 Mean percentages of mammalian specimens from various sites

Bovidae		Equidae	4.4%
(of which 15.1% are		Suidae	3.6%
<i>Madoqua</i> , dik-dik)	43%	Proboscidea	3.4%
Lagomorpha	14.4%	Rodentia	3.3%
Giraffidae		Carnivora	3.1%
(including both a large			
and small species, as			
well as <i>Sivatherium</i>)	11.2%		
Rhinocerotidae	9.7%		

Avifauna, Cercopithecidae, Hominidae and Pedetinae were omitted from this list.

Deeply worn game tracks or pathways can be seen crossing the footprint areas at two sites. These were clearly used repeatedly by animals to reach some objective and on modern behavioural patterns it is likely that they were made by the game going to and from water holes.

Only a proportion of the animals represented in the fossil prints have been identified so far. Investigation of present-day game tracks in National Parks is underway to provide comparative information. In general, however, the fossil record agrees well with the footprints. A Musukuma tracker was employed to assist in identifications. He assisted considerably in identifying to family and generic levels, particularly in the case of Bovidae, which are commonly represented in both the footprint and fossil records.

A partial breakdown of the mammalian specimens recovered from the Laetoli Beds in 1975 was published¹ and may usefully be given here. Additional material was recovered in 1976 and 1977, but the overall proportions of various groups remain close to the first figures (Table 1).

The footprints that have been recorded are briefly described below, with notes on the fossil material where it seems to be related.

Diplopoda

A single track approximately 20 cm long is known at site (A). Fossil record: a small fragment of fossilised centipede was found at Locality 4.

Avifauna

- (1) *Struthio* sp. Two isolated prints at site (A).
- (2) *Phasianidae*, cf. Guinea fowl. Numerous tracks occur at all sites, generally in trails of four or more. They compare closely with tracks of the living helmeted Guinea fowl, common in the Laetoli area today. Average length of eight fossil prints 62 mm, of nine modern prints 60 mm.
- (3) Similar but smaller tracks, averaging 45 mm in length, possibly of francolin.

Fossil record: numerous fragments of ostrich eggshell are known but no skeletal remains. Clutches of eggs comparable in size to those of modern Guinea fowl have been found at Locality 10.

Primates

Cercopithecidae: Tracks are known at three localities.

At site (C) there is a single trail comprising six hind foot prints with a digit protruding to one side. Each of these prints is accompanied by a roughly circular impression, always to the left (Fig. 5). When first discovered these prints were interpreted as knuckle impressions, but more thorough cleaning has revealed traces of the palms of the hands and they are undoubtedly prints of the forefeet. In the hind feet the longest digit is central and the prints range in length from 20.1 to 14.7 cm with an average of 17 cm. The width varies from 10.9 to 8.1 cm with an average of 9.9 cm (excluding the great toe). Stride length varies from 34 to 46 cm with an average of 41 cm. (Stride is here interpreted as the distance between the posterior margin of successive heel prints of the same foot.)

The second trail is at site (D). It was made by a single animal and is 4 m long. There are prints of both hind and forefeet. All are lightly imprinted on a surface which was clearly wet and slippery when the animal walked over it. The average length of the hind prints is 14.5 cm and of the forefeet prints 11 cm. Stride length averages 27.7 cm. These prints are not only smaller than those at site (C) but are relatively broader, with very narrow heel impressions.

At site (F), in fossil Locality 10, there are at least four sets of tracks going in slightly different directions, as do the tracks of present-day baboons when they move in a troop. The average measurements of the hind feet in each of the four trails range from 15.2 to 10 cm and of the forefeet prints from 7.6 to 4 cm (excluding the great toe).

Fossil record: a number of cercopithecoid mandibles and teeth and a few postcranial fragments have been provisionally attributed to *Papio* sp. and a colobine. Most are as small or even smaller in size than those of a living female baboon, but two mandibular fragments, a calcaneum and the distal ends of a humerus and femur are considerably larger than in any living baboon and compare in size with *Theropithecus oswaldi*. The difference in size between the prints described above is compatible with that of the known fossils, although age and sex differences are factors to be considered.

Hominidae: Three trails believed to be hominid are known at sites (A) (Fig. 6) and (G) (Figs 7, 8), in fossil Localities 6 and 8. (1) At site (A) there are five prints in a trail 1.5 m long (Fig. 6). Natural erosion has almost entirely exposed two of the prints, but the remaining three are still filled with matrix of the overlying deposits. The exposed prints are short and broad, 15.5 cm long and 10.5 cm wide. The stride is also short with an average length of 31 cm. The gait was somewhat shambling, with one foot crossing in front of the other. Unlike the cercopithecoid prints, the longest digit is the great toe, situated as in the human foot.

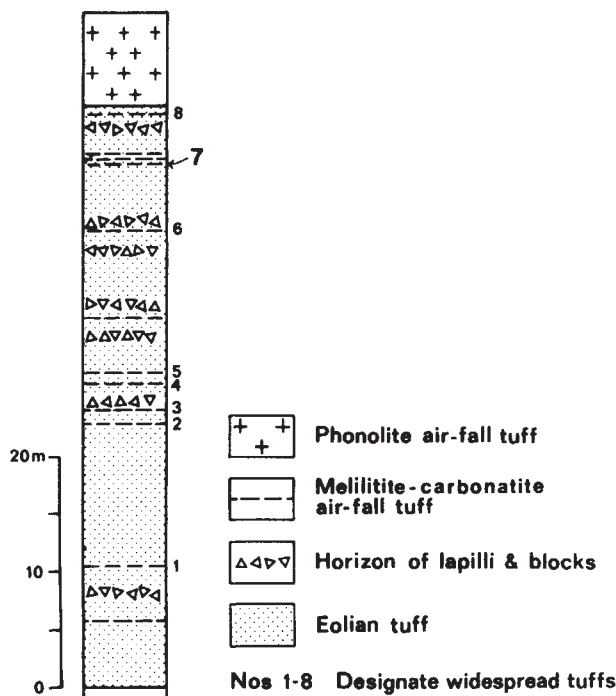


Fig. 3 Columnar section of the upper unit of the Laetoli Beds at Locality 1 showing air-fall tuffs and horizons of lapilli and blocks. The present report is based on studies of the lower part of Tuff 7, termed the Footprint Tuff. Tuffs 6, 7 and 8 were designated Tuffs a, b and c by Leakey *et al.*¹. The phonolite tuff was designated Tuff d.

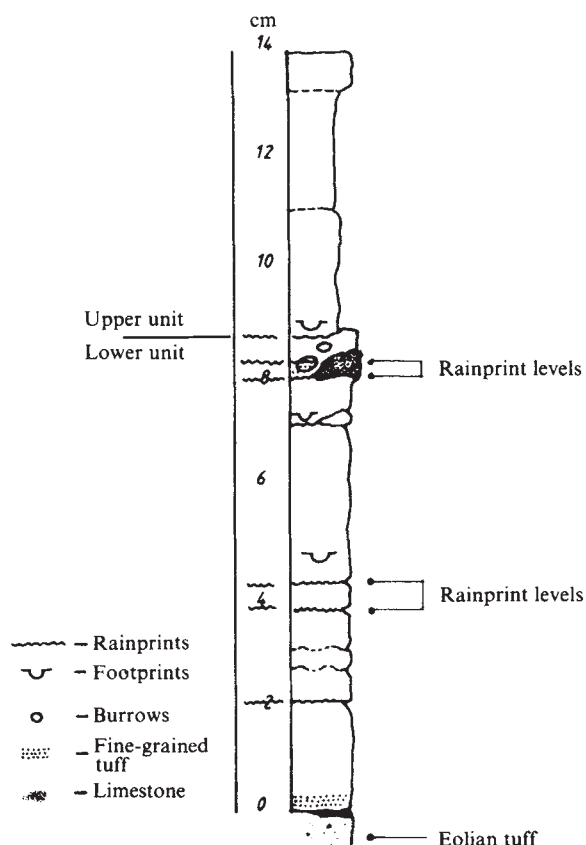


Fig. 4 Generalised columnar section for footprint tuff at site A.

It has been suggested that these prints might have resulted from the superposition of a hind on forefoot impression, or vice versa, either of a quadrupedal animal or a knuckle-walking primate. Careful examination of the original prints *in situ* reveals no indication of superposition, while the last interpretation invokes the hypothetical existence of an animal which is not present in over 5,000 fossil specimens.

(2) At site (G) there are trails left by two individuals travelling north (Figs 7–9). The trails are parallel and ~25 cm apart, too close for the hominids to have walked abreast. They followed the same line or pathway but it is possible that they did not pass by at the same time as there is a noticeable difference in the conditions of the two sets of prints. Those of the smaller individual are sharp and well defined, indicating a firm, compact surface, whilst those of the larger individual, with one notable exception, are blurred at the edges and enlarged, as would be the case if the surface had been dry and dusty. At one point the smaller individual appears to have paused and made a half-turn to the left before continuing in a northerly direction.

In a number of prints the original surface in which they were made has been eroded, leaving only indentations in the underlying deposits. Thus, the measurements of these prints do not accurately reflect the dimensions of the original impressions and are not included.

The site is now being excavated and the trails have so far been found to extend for a distance of 23.54 m. Trail 1 contains 22 prints and Trail 2 contains 12 prints. The average length/breadth of the prints in Trail 1 is 18.5 × 8.8 cm and in Trail 2 is 21.5 × 10 cm. Average stride length in Trail 1 is 38.7 cm and in Trail 2 is 47.2 cm. Note that the longitudinal arch of the foot is well developed and resembles that of modern man, and the great toe is parallel to the other toes (Figs 7, 8 and 9).

Fossil record: two mandibles, parts of 2 maxillae, partial deciduous and permanent dentitions as well as a partial infant

skeleton and a number of isolated teeth have been recovered from the Laetoli Beds. They bear considerable resemblances to the material collected from the Afar in Ethiopia, although the Ethiopian material is substantially later.

Leporidae

Innumerable tracks of hares occur at all sites.

Fossil record: mandibles and other remains attributed to *Serengetilagus* sp. are abundant.

Carnivora

(1) Viverridae, indet. Series of small carnivore prints with non-retractile claws occur at all sites. They are generally lightly imprinted and rather faint. Average length/breadth 34 × 27 mm. Other small, rounded prints without claw marks, suggest genets. Average length/breadth 28 × 27 mm.

Fossil record: a variety of Viverridae are known among the fossils, some were described by Dietrich³, those from the recent collections are being studied by Mme G. Petter.

(2) Hyenidae. Hyenas have left a series of well-defined and relatively long trails containing large numbers of prints. Eight trails are known and two gaits are represented. Subject to research on living hyenas, these appear to be a walk and a slow canter or lope. There is uniformity in size, depth and stride within each of the four trails measured, although it is evident that the animals varied in individual size. Average length/breadth/depth measurements:

Trail C.22 (19 prints)	122 × 94 × 12 mm
Trail C.27 (30 prints)	125 × 102 × 22 mm
Trail C.31 (11 prints)	102 × 87 × 13 mm
Trail C.39 (18 prints)	111 × 100 × 18 mm

Fossil record: in a preliminary report, M. G. Leakey noted the existence of three species of hyena from the Laetoli Beds: (1) *Hyaena bellax* (Ewer 1954); (2) *Hyaenictis* cf. *preforlex* (Hendey 1974); and (3) *Lycyaena* sp.

(3) Felidae. One trail of 12 prints at site (A), and several isolated prints appear to correspond in size to those of a serval



Fig. 5 Print of cercopithecoid fore and hind foot at site C.



Fig. 6 Hominid footprints at site A.

cat. Average length/breadth/depth $65 \times 52 \times 13$ mm. Average length of stride 29 mm.

Fossil record: remains comparable to *F. serval* and *F. caracal* have been noted in the 1974–76 collections.

(4) *Machairodontinae*. Two prints at site (A), measuring 134×115 mm and 110×115 mm probably belong to a large sabre-tooth cat, as no other felid of comparable size occurs in the fossil material.

Fossil record: The presence of a large sabre-tooth cat, cf. *Homotherium* is indicated by two teeth.

Proboscidea

The proboscidean prints appear to belong to *Loxodonta exoptata* with the exception of one particularly well-preserved print in a trail of four at site (C) in which the phalanges and metapodials were more nearly vertical than in other known proboscidean prints and may be of a *Deinotherium*. The *Loxodonta* prints include a number made by juvenile animals as well as some that are usually large by present-day standards. The average for 15 measured prints, mostly adult, is $420 \times 346 \times 34$ mm.

Fossil record: both *Loxodonta exoptata* and *Deinotherium* cf. *bozasi* occur in the Laetoli Beds, but the latter is relatively scarce. A high proportion of the *Loxodonta* teeth are from juvenile animals.

Equidae

Only two equid trails are known. They have only recently been discovered and have not yet been fully studied. Both are at site (G), one on either side of the hominid trails but travelling in an opposite direction, to the south. The best preserved trail is 4.97 m long and contains 15 prints, nine of which can be measured. They range in length from 9.9 to 7.8 cm with an average of 8.6 cm and in width from 10.6 to 8.3 cm with an average of 8.8 cm. The animal appears to have changed gait during this trail.

Fossil record: numerous teeth, postcranial material and some incomplete mandibles have been found. All can be attributed to *Hipparion* sp.

Rhinocerotidae

Both *Ceratotherium* and *Diceros* must be represented among the many prints of rhinoceros, but no distinguishing features have been observed to date except on the basis of size. A trail at site (A) is one of the longest known, measuring 22 m in length. It contains 31 prints, 23 of which are double, with the hindfoot superimposed on the front. At the western end of the trail, where the animal changed gait, the prints become single and are irregularly spaced. Comparison with modern prints of *Diceros bicornis* at Olduvai show very close similarity. Average length/breadth/depth of double prints in trail: $416 \times 273 \times 31$ mm. Single prints, forefoot $246 \times 248 \times 25$ mm, hindfoot

$256 \times 228 \times 37$ mm. Isolated single fore and hind prints in the adjacent game trail are unusually large and may be of *Ceratotherium*. They measure $285 \times 310 \times 42$ mm and $400 \times 270 \times 33$ mm respectively.

Fossil record: on the basis of the early collections only *Ceratotherium* was believed to be present in the Laetoli fauna. But a skull of *Diceros* has now been found, as well as numerous teeth and some postcranial material.

Chalicotheriidae

Two Chalicotheres prints were found at site (C). They are deeply indented and measure $250 \times 155 \times 63$ mm and $243 \times 110 \times 46$ mm. The prints comprise impressions of three digits and of the palm. The digits have left symmetrical rounded grooves and the emplacement for a claw can be seen on one. The prints are 1.30 m apart.

Fossil record: a few specimens which can be referred to *Ancylotherium* cf. *hennigi* have been recovered. They include a calcaneum, astragalus and some phalanges.

Suidae

Thirteen suid prints at site (A) have been measured. They are comparable in size to prints of the living warthog. Average length/breadth/depth measurements are $55 \times 47 \times 11$ mm.

Fossil record: only two Suidae are known from the Laetoli Beds, *Potamochoerus* sp. and *Notochoerus euilus*. *Hylochoerus* and *Phacochoerus* were previously believed to be present in the early fauna, but during 1974–77 have only been found in the more recent deposits. On size, the prints are probably of *Potamochoerus*, as *Notochoerus* was a larger animal than the living warthog.

Giraffidae

(1) Three trails made by single animals are known. They consist of 6, 7 and 11 prints respectively. There are also 19 other prints attributed to giraffe. In one trail, the animal has dragged its feet after each step and left scuffed grooves up to 96 cm long. Average length/breadth/depth measurement for the prints in the trails are $190 \times 151 \times 29$ mm, $202 \times 144 \times 10$ mm, $211 \times 150 \times 17$ mm and $205 \times 150 \times 28$ mm. Averages for the isolated prints are $208 \times 155 \times 19$ mm.

Fossil record: the prints are of similar size to those of the modern giraffe and can be attributed to *G. jumae* which occurs as a fossil.

(2) Small giraffe cf. *G. stillei*. There are no prints which can positively be allocated to this animal, although its fossil remains are by far the most common giraffid. However, there are many prints, including three trails, which were identified as 'eland' by the tracker. No eland is known in the fossil fauna, nor is there any other bovid in the collection of a size suitable to have made these prints. For the present, it seems justifiable to assign these prints to the small giraffe. They are abundant at site (A), where there are three trails consisting of 21, 14 and 5 prints, as well as 72 isolated prints. The average length/breadth/depth measurements for the prints in the three trails are $140 \times 107 \times 14$ mm, $139 \times 104 \times 13$ mm and $128 \times 94 \times 16$ mm.

Fossil record: if the prints are correctly identified, they would belong to the animal originally named *Okapia stillei*, now known as *Giraffa stillei*.

(3) *Sivatherium*. No prints could be identified. The fossils consist mostly of isolated teeth and foot bones.

Bovidae

(1) Bovini cf. *Simatherium kohllarseni*. These prints consist of large, rounded and generally deeply indented tracks resembling those of the living African buffalo. They are clearly made by Bovini and can be attributed to *S. kohllarseni*, as this is the only bovine in the fossil record. The prints are represented by four trails containing 16, 12, 10 and 5 tracks, as well as 40 additional



Fig. 7 Dual trail of hominid footprints at site G.

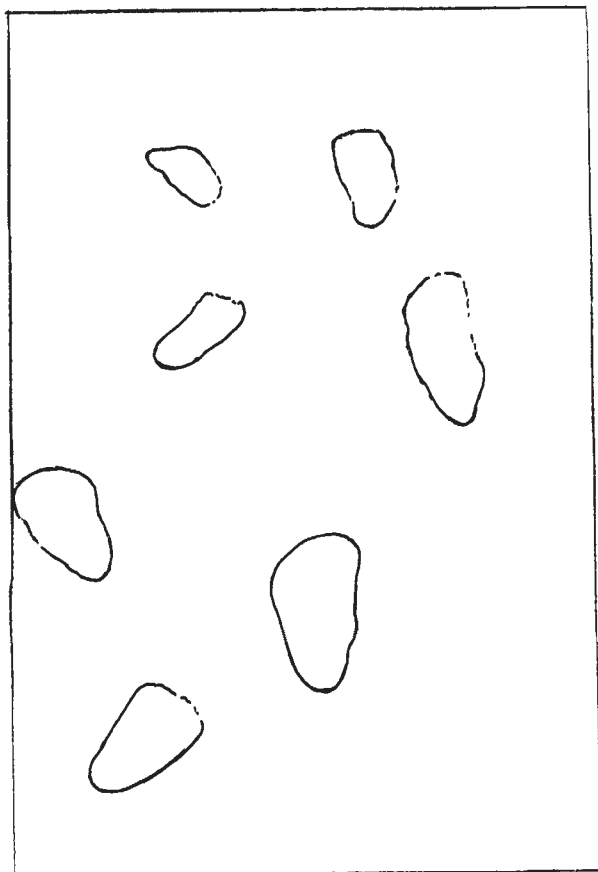


Fig. 8 Outline of footprints shown in Fig. 7.

prints, either single or in pairs. The average length/breadth/depth measurements for the 43 prints in the trails are $185 \times 149 \times 25$ mm.

Fossil record: a number of horn cores and teeth have been found, including a cranium associated with both horn cores. (2) *Hippotragus* sp. Seven single prints from sites (A) and (C) were identified by the tracker as 'roan antelope'. They are characterised by widely splayed, elongate hoof-marks. The average length/breadth/depth measurements are $109 \times 95 \times 16$ mm.

Fossil record: three horn cores and some teeth, collected during 1975 are believed to be a hippotragine and have



Fig. 9 Left footprint of larger individual in dual trail at site G.

provisionally been attributed to *Praedamalis deturi*. A horn core and teeth in the earlier collections may also belong to this species which is smaller than the living roan antelope.

(3) Alcelaphini. Eighteen prints at site (A) and two at site (C) were identified by the tracker as hartebeest. The prints at site (A) are both smaller and shallower than those at site (C). Average length/breadth/depth for the former are $80 \times 60 \times 14$ mm and for the latter $103 \times 80 \times 23$ mm.

Fossil record: *Parmularius* sp. has been identified on a frontlet with horn cores, teeth and other fragmentary horn cores. There is also a cranium collected in 1959 which has been attributed to a larger species of alcelaphine. A third species may also be represented.

(4) Small antelopes and gazelles. Prints of dik-dik (*Madoqua*) are rare although dik-dik are the most abundant single species of fossil Bovidae. This anomaly may be explained by the fact that dik-dik are one of the Bovidae who do not require to drink

water, subsisting on moisture from vegetation, while tracks of other Bovidae were probably made going to or from water holes.

Fossil record: *Neotragini*? and *Raphicerus* sp. (Steenbuck) are provisionally identified. *Madoqua* is very abundant. *Antilopini*: the gazelle appears to be *G. janenschii*.

Conclusions

The greater part of the fossil fauna from the Laetoli Beds is recorded in the fossil tracks. In all, over 20 taxa are represented. The preservation of the footprints can be attributed to an unusual and possibly unique combination of climatic, volcanic and mineralogic conditions. The available evidence indicates that the episode took place during a brief period, probably during the onset of a single rainy season which happened to coincide with the eruption of light ash showers from the nearby volcano Sadiman.

The locomotor pattern displayed by the trails of hominid footprints is still under examination but it is immediately evident that the Pliocene hominids at Laetoli had achieved a fully upright, bipedal and free-striding gait; a major event in the evolution of man which freed the hands for tool-making and eventually led to more sophisticated human activities. Moreover, evidence supplied by cranial parts of the somewhat later but related hominid fossils from the Afar in Ethiopia (dated between 2.6 and 3 Myr) indicates that bipedalism outstripped

enlargement of the brain. To have resolved this issue is an important step in the study of human evolution, as it has long been the subject of speculation and debate.

With the hands free and available for purposes not connected with locomotion it is perhaps surprising that no form of artefact has been found. But the concept of tool-making may well have been beyond the mental ability of these small-brained creatures. Any 'tools' or weapons used must have been solely of perishable materials as the Laetoli Beds are devoid not only of artefacts but of all stones other than volcanic ejecta.

Further exploration of sites and analysis of material will continue in 1979, but it is evident that Laetoli will give an unique perspective into hominid environment during Pliocene times.

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The chick ovomucoid gene contains at least six intervening sequences

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A 15-kilobase pair EcoRI chick DNA fragment, containing both the termination codon UGA and the 5'-portion of the structural ovomucoid gene, has been cloned in λ phage Charon 4A by in vitro packaging. Restriction mapping and electron microscopic analyses of this cloned DNA have revealed that the structural ovomucoid gene sequences are separated by at least six intervening sequences.

OVOMUCOID is a major egg-white protein, and its synthesis in the chick oviduct is regulated by the steroid hormones oestrogen and progesterone^{1–3}. Using a specific hybridisation probe comprised of a complementary DNA synthesised from purified ovomucoid messenger RNA, it has been demonstrated that the steroid-mediated induction of ovomucoid synthesis in the chick oviduct is preceded by the accumulation of its messenger RNA⁴. We have recently succeeded in the isolation and amplification of the structural ovomucoid gene by molecular cloning⁵. Using the cloned ovomucoid DNA as a specific hybridisation probe, we have shown that the kinetics of hormonal induction of ovomucoid mRNA accumulation in the chick oviduct is similar to that of ovalbumin mRNA^{6–9}. To study the mechanisms by which steroid hormones regulate the expression of these oviduct genes, we attempted to define their sequence organisation within genomic chick DNA. We have recently reported the cloning of various *EcoRI* fragments of the natural ovalbumin

gene^{10–13}. Detailed analysis of these cloned DNA fragments have shown that the structural ovalbumin gene is separated into at least eight portions by seven intervening sequences^{11,13,14}. The discovery of intervening sequences within eukaryotic genes has introduced a new level of complexity in the elucidation of the mechanism of gene expression. To substantiate the pattern for genetic organisation, we have attempted to clone the natural ovomucoid gene so that we might establish the sequence organisation of this gene.

The recombinant plasmid pOM100

Ovomucoid complementary DNA (cDNA_{om}) was previously synthesised from a partially purified ovomucoid mRNA preparation and cloned in the *Pst*I site of pBR322 (ref. 5). Several recombinant clones have been demonstrated to contain inserted ovomucoid cDNA sequences by direct DNA sequencing. One of these recombinant plasmids, designated pOM100, contains about 650 base pairs of DNA, including the 3'-portion of the mRNA, and lacks about 125 base pairs at the 5'-terminus of the structural ovomucoid gene. A preliminary structure of the cloned ovomucoid cDNA (Fig. 1) has been established by restriction mapping⁵ and DNA sequencing (J.F.C., unpublished). It contains a *Kpn*I site very close to the 5'-end of the inserted DNA. There is an unique *Hinc*II site and two cleavage sites each for *Pst*I and *Hae*III within the peptide coding portion of the gene. The ovomucoid structural gene also contains 138 base pairs of untranslated region at the 3'-terminus following the termination codon and there are unique *Eco*RI and *Bam*HI sites

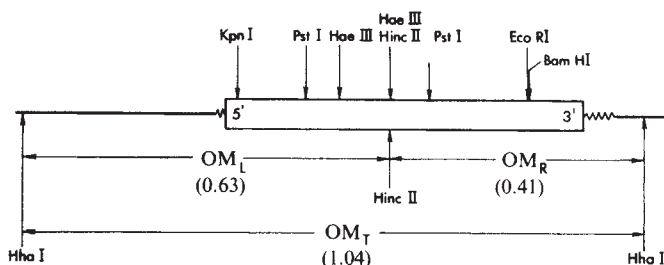


Fig. 1 Structure of pOM100 and the preparation of ovomucoid DNA fragments as specific hybridisation probes. □, Ovomucoid DNA insert; —, pBR322 DNA; wavy line, dA-dT linkers. The various restriction endonuclease sites of the ovomucoid DNA insert are as indicated by the arrows. The numbers in parentheses indicate the size of the fragments in kilobases. 300 µg of pOM100 DNA was digested with *HhaI* (BRL) in standard conditions. The digestion products were separated by electrophoresis in a 1% preparative agarose gel. The 1.04-kilobase fragment (OM_T) was recovered from the gel by diffusion as described previously³⁶ and was further digested with *HincII* into two fragments of 0.63 (OM_L) and 0.41 (OM_R) kilobases, respectively. These fragments were again isolated by preparative gel electrophoresis. OM_T , OM_L and OM_R were labelled with [α -³²P]dCTP by nick translation to a specific radioactivity of about 2×10^8 c.p.m. per µg (ref. 15).

located in this region. The cloned ovomucoid DNA is not cleaved by the restriction enzymes *HhaI*, *HindIII*, *AvaI* or *TaqI*.

As the cloned ovomucoid DNA does not contain any *HhaI* cleavage site, a DNA fragment containing the entire inserted DNA fragment was prepared by *HhaI* digestion of pOM100 followed by preparative agarose gel electrophoresis (Fig. 1). This 1.04-kilobase DNA fragment (OM_T) was further digested with *HincII* into two DNA fragments containing the left (OM_L) and right (OM_R) halves of the cloned ovomucoid DNA. These DNA fragments were again separated from each other by preparative agarose gel electrophoresis and used as specific hybridisation probes after labelling by nick translation¹⁵.

Identification of chick DNA fragments containing the ovomucoid gene

Total chick DNA was digested with *EcoRI* and subjected to agarose gel electrophoresis. A large number of DNA fragments, heterogenous in size, can be visualised as a broad banding pattern in the gel after staining with ethidium bromide (Fig. 2A, lane a). The DNA fragments in the gel were transferred onto a nitrocellulose filter by the method of Southern¹⁶. The filter was treated according to the procedure of Denhardt¹⁷, and those DNA fragments on the filter containing structural ovomucoid gene sequences were identified by hybridisation with ³²P- OM_T followed by autoradiography¹⁸ (Fig. 2B, lane a). It is evident from this experiment that the structural ovomucoid gene sequences are contained within two *EcoRI* DNA fragments of 15 and 7 kilobases. The separation of the structural gene sequences into two *EcoRI* DNA fragments was not unexpected, as there is an *EcoRI* cleavage site within the structural gene. The order of these two DNA fragments was established by hybridising total *EcoRI*-digested DNA with OM_L and OM_R separately. The 15-kilobase DNA fragment hybridised with both probes, whereas the 7-kilobase DNA fragment hybridised with OM_R only (data not shown). Thus, the 15-kilobase DNA fragment contains the 5'-portion of the ovomucoid gene.

Cloning of the natural ovomucoid gene

The 15-kilobase DNA fragment was enriched from total *EcoRI*-digested chick DNA by preparative agarose gel electrophoresis. The gel was cut into 3-mm slices and DNA recovered from each gel slice was assayed for ovomucoid gene content by analytical gel electrophoresis followed by Southern hybridisation using

³²P- OM_T . The ethidium bromide staining pattern and autoradiogram of these individual DNA fractions are shown in lanes b-e of Fig. 2A and B, respectively. It is evident that the DNA fraction displayed in lane c is enriched for the 15-kilobase ovomucoid DNA fragment. This DNA fraction was thus used to clone the ovomucoid gene.

λ Phage Charon 4A contains three *EcoRI* cleavage sites and yields four DNA fragments on digestion with *EcoRI* (Fig. 2A, lane f). The two upper phage DNA arms were separated from the middle *EcoRI* DNA fragments by preparative agarose gel electrophoresis, and allowed to ligate in the presence of the DNA fraction enriched for ovomucoid DNA using T4 DNA ligase. The ligation mixture was used for cloning in a P3 facility by *in vitro* packaging according to the method of Sternberg *et al.*¹⁹ as modified by Faber, Kiefer and Blattner (personal communication). Briefly, λ lysogens were induced and cell extracts were made by alternate freezing and thawing and by sonication. The two protein extracts were mixed with purified λ A protein and incubated at 37 °C for 60 min and 5 µl of the ligation mixture in a final volume of 0.25 ml. The packaging mixture was plated in 12-µl aliquots in soft agar on 20 agar plates. Approximately 20,000 phage plaques were generated in this experiment. These phages were screened for the recombinant phage containing the ovomucoid gene by hybridisation with the OM_T probe using an amplification procedure as described previously¹⁰. Three phage plaques gave positive signals on the autoradiograms in this test. These phages were cultured individually and their DNA prepared. *EcoRI* digestion of DNA from any of the three phages produced two DNA fragments corresponding to the arms of Charon 4A and an additional 15-kilobase DNA fragment (Fig. 2A, lane g), which hybridised with the ovomucoid structural sequence OM_T on Southern analysis (Fig. 2B, lane g). This 15-kilobase DNA fragment of the natural ovomucoid gene was subsequently recloned in pBR322 in *Escherichia coli* χ 1776 and was designated pOM15.

Restriction mapping of the cloned 15-kilobase ovomucoid DNA

The 15-kilobase ovomucoid DNA (OM_{15}) was excised from pBR322 DNA by *EcoRI* digestion of the recombinant plasmid pOM15 followed by preparative agarose gel electrophoresis. The excised ovomucoid DNA was further digested with a variety of other restriction endonucleases and those DNA fragments containing the left and right halves of the structural ovomucoid

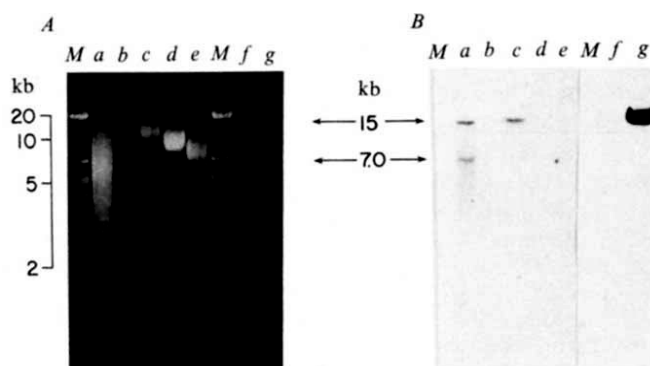


Fig. 2 Agarose gel electrophoresis of various DNA fractions and identification of ovomucoid DNA sequences by Southern¹⁶ hybridisation. A Shows the ethidium bromide stain of the gel and B the corresponding autoradiogram after the DNA was transferred from the gel to a nitrocellulose filter and hybridised to ³²P- OM_T . Lane M: 1 µg of *EcoRI*-digested wild-type λ phage DNA; lane a: 15 µg of *EcoRI*-digested total chick DNA; lanes b-e: 1.0 µg each of *EcoRI*-digested chick DNA extracted from various gel slices of a preparative gel; lane f: 0.5 µg of the *EcoRI*-digested Charon 4A λ DNA; lane g: *EcoRI*-digested DNA from one of the three recombinant phages that hybridised with ³²P- OM_T .

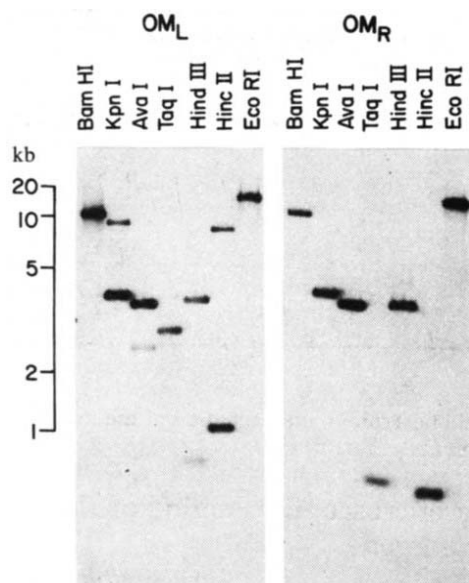


Fig. 3 Autoradiogram of 1% agarose gel containing OM15 DNA after digestion with various restriction endonucleases and Southern hybridisation. OM15 DNA was extracted from *EcoRI*-digested pOM15 by preparative agarose gel electrophoresis. 100 ng of purified OM15 DNA were digested by respective restriction endonucleases as indicated above each lane in the autoradiogram. Electrophoresis was carried out in 1% agarose gels and DNA was transferred to nitrocellulose. Hybridisation was carried out with ^{32}P -labelled OM_L (left panel) and ^{32}P -labelled OM_R (right panel), respectively.

gene sequences were identified by agarose gel electrophoresis followed by Southern hybridisation with the OM_L and OM_R probes, respectively. Some of the data are shown in Fig. 3 and summarised in Table 1. These data were used to construct a preliminary map of the natural ovomucoid gene as shown in Fig. 4. As expected, the OM15 DNA hybridised with both probes (Fig. 3). *HincII* digestion of OM15 DNA resulted in two DNA fragments of 8.0 and 1.1 kilobases that hybridised with OM_L and a unique 0.7-kilobase DNA fragment that hybridised with OM_R but not OM_L (Fig. 3). Thus, there is a *HincII* site (*HincII*_c) 0.7 kilobases to the left of the *EcoRI*_b site (Fig. 4). By virtue of the method used to prepare the two probes, the *HincII*_c site must be the unique *HincII* cleavage site present in the structural ovomucoid gene (Fig. 1). Limited sequence analysis has also established that the *EcoRI*_b site is the one present in the struc-

tural ovomucoid gene (data not shown). The unique *HincII* and *EcoRI* sites in the structural gene, however, are separated by only 0.2 kilobases of DNA. Thus, it seems that these two sites may be separated by an intervening sequence in genomic DNA. Furthermore, there should be a *HincII* site (*HincII*_a) 9.1 kilobases to the left of *HincII*_c. The *HincII*_b site, however, could be either 8.0 or 1.1 kilobases to the left of the *HincII*_c.

BamHI digested the OM15 DNA into a 10-kilobase DNA fragment hybridisable with both OM_L and OM_R (Fig. 3). In addition, there is a 0.3-kilobase fragment hybridisable only with OM_R, and resistant to *HincII* digestion (Table 1). Thus, there should be a *BamHI* site (*BamHI*_b) 0.3 kilobases to the left of *EcoRI*_b, and an additional *BamHI* site (*BamHI*_a) 10 kilobases to the left of *BamHI*_b (Fig. 4). The presence of these two *BamHI* sites predicts that a *HincII* digestion of the *BamHI* DNA fragments would result in a 8.0 and a 1.1 kilobase DNA fragment that would hybridise with OM_L only, and that the 0.7-kilobase *HincII* DNA fragment would be digested by *BamHI* into two fragments of 0.4 and 0.3 kilobases, respectively. These are exactly the results observed (Table 1). As there is no *BamHI* cleavage site between the *HincII* and *EcoRI* sites in the structural gene, these results confirm that there must be at least one intervening sequence separating these two restriction sites in the structural ovomucoid gene.

HindIII digested OM15 DNA into a 3.5-kilobase DNA fragment hybridisable with both OM_L and OM_R probes, as well as a 0.9-kilobase DNA that hybridised with OM_L only (Fig. 3). Thus, there should be a *HindIII* site (*HindIII*_b) 3.5 kilobases to the left of *EcoRI*_b, and an additional *HindIII* site (*HindIII*_a) 0.9 kilobases to the left of *HindIII*_b (Fig. 4). Indeed, the 3.5-kilobase DNA is cleaved by *HincII* into a 1.1 and a 0.7 kilobase fragment hybridisable with OM_L and OM_R, respectively, which are the same DNA fragments observed by digestion of OM15 DNA with *HincII* alone (Table 1). This experiment further suggests that the *HincII*_b site is 1.1 kilobases to the left of *HincII*_c and that there is no structural gene sequence between *HindIII*_b and *HincII*_b. In addition, there should be some structural gene sequence within the 0.9-kilobase *HindIII* DNA fragment (Fig. 4). The fact that the structural gene contains no *HindIII* cleavage site suggests that there may be more intervening DNA sequences within the natural ovomucoid gene.

KpnI digested the OM15 DNA into a 3.7-kilobase fragment hybridisable with both OM_L and OM_R and an additional 8.5-kilobase DNA fragment hybridisable only with OM_L (Fig. 3). Hence, there should be a *KpnI* site (*KpnI*_b) 3.7 kilobases to the left of *EcoRI*_b and another *KpnI* site (*KpnI*_a) 8.5 kilobases to the

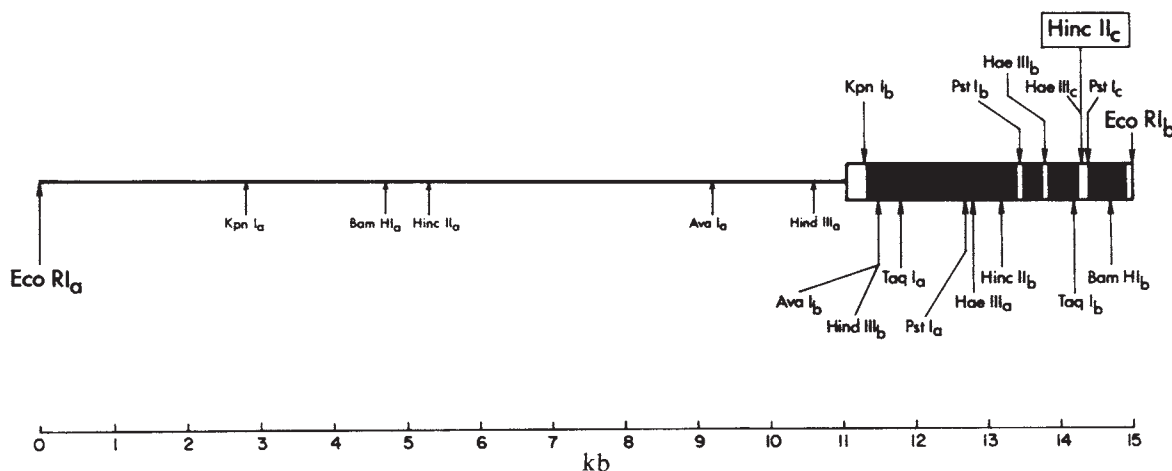


Fig. 4 Structural organisation of the natural ovomucoid gene (OM15). Ovomucoid structural sequences are represented by $\square$, intervening sequences by $\blacksquare$, and flanking DNA sequences by — . The arrows indicate various restriction endonuclease sites on the ovomucoid gene. Those above the schematic drawing indicate restriction sites present in the structural ovomucoid gene and those below are sites within the intervening or flanking DNA sequences. There are additional restriction sites present in the left-hand portions of OM15 DNA, but their positions have not been mapped and are therefore not shown.

Table 1 Sizes of ovomucoid structural sequence-containing fragments after restriction endonuclease digestion of OM15

	Size after digestion (kilobases)								Probe used
	<i>Eco</i> RI	<i>Hind</i> III	<i>Bam</i> HI	<i>Taq</i> I	<i>Ava</i> I	<i>Kpn</i> I	<i>Hae</i> III	<i>Pst</i> I	
– <i>Hinc</i> II	15.0	3.5	10.0	2.4	3.5	8.5	1.0	0.75	OM _L
		0.9			2.3	3.7	0.5	0.75	
+ <i>Hinc</i> II	8.0	1.1	8.0	1.1	2.3	6.0	0.6	0.7	OM _L
	1.1	0.9	1.1		1.1	1.1	0.5	0.3	
– <i>Hinc</i> II	15.0	3.5	10.0	0.75	3.5	3.7	0.7	0.65	OM _R
			0.3						
+ <i>Hinc</i> II	0.7	0.7	0.4	0.7	0.7	0.7	0.7	0.65	OM _R
			0.3						

left of *Kpn*I_b. Double digestion of OM15 DNA with *Kpn*I and *Hinc*II yielded a 0.7-kilobase fragment hybridisable with OM_R as expected, as well as two DNA fragments of 6.0 and 1.1 kilobases that hybridised with OM_L as predicted from the map (Fig. 4). Thus, the *Kpn*I_b site seems to be the one that is present towards the 5'-terminus of the structural ovomucoid gene (Fig. 1). Because this *Kpn*I site is separated from the *Hinc*II_c site by a *Hind*III site in genomic DNA but not in the structural gene, there should be a second intervening sequence separating these sites in the natural ovomucoid gene.

The OM15 DNA was digested by *Hae*III into a unique 0.7-kilobase DNA fragment hybridisable with OM_R and resistant to *Hinc*II digestion (Table 1). Hence, there is a *Hae*III site (*Hae*III_c) about 0.7 kilobases to the left of *Eco*RI_b, which could be one of the two *Hae*III sites located near the unique *Hinc*II site in the structural gene (Fig. 1). As there are two *Hae*III fragments of 1.0 and 0.5 kilobase that hybridised with OM_L, there should be a *Hae*III site (*Hae*III_a) 1.5 kilobases to the left of *Hae*III_c. Furthermore, the 0.5-kilobase *Hae*III fragment seem to be resistant to *Hinc*II, whereas the 1.0-kilobase fragment is cleaved by this enzyme to a 0.6-kilobase fragment hybridisable with OM_L (Table 1). Thus, the *Hae*III_b site should be located 0.5 kilobases to the left of *Hae*III_c. Otherwise, the 1.0-kilobase *Hae*III DNA fragment would be resistant to *Hinc*II and the 0.5-kilobase fragment would be digested to a 0.4- and a 0.1-kilobase fragment. Furthermore, this *Hae*III_b site may be the second *Hae*III site present in the structural gene (Fig. 1), as both the 0.5- and 1.0-kilobase *Hae*III DNA fragments hybridised with OM_L. By similar analysis, a *Taq*I site (*Taq*I_b) has been located between the *Hae*III_b and *Hae*III_c sites (Fig. 4). As the *Hae*III sites in the structural gene are not separated by a *Taq*I site, there may be a third intervening sequence within the ovomucoid gene.

*Pst*I digested the OM15 DNA into a unique 0.65-kilobase fragment hybridisable with OM_R, and resistant to further digestion by *Hinc*II (Table 1). Hence, a *Pst*I site (*Pst*I_c) should be located 0.65 kilobases to the left of *Eco*RI_b and could be the *Pst*I site located 0.05 kilobases to the right of the unique *Hinc*II site present in the structural gene (Fig. 1). Although *Pst*I digestion yielded only one 0.75-kilobase band when hybridised with OM_L (Table 1), there must be at least two DNA fragments of the same size because one of the fragments was digested by *Hinc*II into a 0.3-kilobase fragment and the other fragment remained resistant. Hence, there should be two *Pst*I sites, 0.75 kilobases apart, to the left of *Pst*I_c. Moreover, the *Pst*I_b site may be the other *Pst*I present in the structural gene (Fig. 1), because the 0.75-kilobase *Pst*I fragment would have been digested to one of 0.45 kilobases instead of a 0.3-kilobase fragment if *Pst*I_a were the site present in the structural gene instead. The finding that the *Pst*I_b and *Hae*III_b sites are separated by 0.3 kilobases in genomic DNA but by only about 0.07 kilobases in the structural gene (Fig. 1), suggests that there may be a fourth intervening sequence within the ovomucoid gene.

These results indicate that the structural ovomucoid gene sequences are not contiguous and are apparently separated into several portions by multiple intervening sequences within the chick genome. The existence of four intervening sequences as established by limited restriction analyses of the cloned OM15

DNA should be a minimum estimation of the number of intervening sequences.

Electron microscopic mapping of the ovomucoid gene

Although intervening sequences within structural genes can be detected by restriction enzyme mapping, a complete map requires other methods independent of the chance occurrence of restriction sites. Short structural gene regions may be missed due to the relative instability of the hybrids formed during hybridisation, resulting in weak signals on Southern filter analysis. In an attempt to characterise the structure of the ovomucoid gene more fully, hybrid molecules formed between ovomucoid mRNA and the cloned 15-kilobase ovomucoid DNA were examined by electron microscopy.

DNA was thermally denatured and incubated with ovomucoid mRNA in conditions that permit only RNA:DNA hybridisation but not DNA:DNA reassociation. Thus, homologous regions between the mRNA and the cloned DNA would appear as double-stranded structures and between the double-stranded regions, DNA sequences non-homologous with the mRNA would appear as single-stranded loops. Figure 5 shows such a hybrid molecule formed between ovomucoid mRNA and the cloned OM15 DNA. Six intervening DNA loops of various sizes are apparent in this molecule, all of which were present in 10 individual hybrid molecules examined. The relative positioning of these loops was established in a histogram as shown in Fig. 6. All the loop structures occurred at one DNA

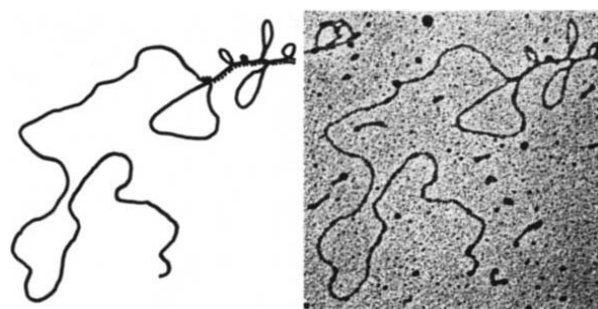


Fig. 5 Electron micrograph and line drawing of a hybrid molecule formed between the ovomucoid gene (OM15) and ovomucoid mRNA. Hybridisation was carried out using $10 \mu\text{g ml}^{-1}$ of OM15 DNA and $20 \mu\text{g ml}^{-1}$ of ovomucoid mRNA in 70% deionised formamide containing 100 mM Tris-HCl, pH 7.6, 10 mM sodium EDTA and 150 mM NaCl. The mixture was heated at 80°C for 5 min to denature the DNA and hybridisation was carried out at 43°C for 2 h. The samples were immediately prepared for electron microscopy as follows. The hybridisation mixture containing 0.1–0.5 μg of nucleic acids was diluted into 100 μl of a solution containing 70% formamide, 0.1 M Tris-HCl, pH 8.4, 0.01 M sodium EDTA and 100 $\mu\text{g ml}^{-1}$ of cytochrome c. The mixture was spread onto a hypophase of distilled water. Samples were collected on collodion-coated 300-mesh copper grids, stained with uranyl acetate, rotary shadowed with platinum-palladium, and examined at 80 kV on a Joel 100 C electron microscope. —, OM15 DNA; ----, mRNA_{om}.

terminus and occupied about one-third of the total length of the OM15 DNA molecule. This observation agrees with the restriction map as shown in Fig. 4. Two additional intervening sequences were detected in these studies. The short intervening sequences *C* and *F* were not detected by restriction mapping analysis.

Comments

We have reported the cloning of the chick ovomucoid gene in λ phage Charon 4A by *in vitro* packaging. The great efficiency of *in vitro* packaging has enabled us to limit purification of the gene to approximately 10-fold by preparative agarose gel electrophoresis and only nanogram quantities of DNA are required. *In vitro* packaging thus makes it possible to eliminate laborious DNA enrichment procedures before cloning.

Four intervening sequences were detected in the ovomucoid gene by limited restriction enzyme mapping. Electron microscopic analysis of RNA:DNA hybrids between ovomucoid mRNA and the 15-kilobase cloned ovomucoid gene fragment showed six loops corresponding to intervening sequences. Both methods have advantages in locating intervening sequences. Restriction mapping is more quantitative in that fragment sizes

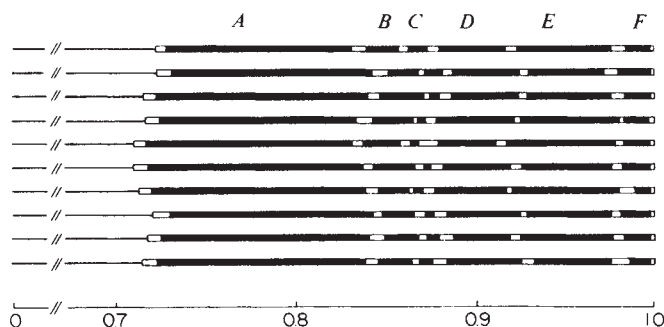


Fig. 6 Histogram of 10 individual hybrid molecules formed between OM15 DNA and ovomucoid mRNA in conditions described in the legend to Fig. 5. The molecules were measured using a map measure, and the sizes and positions of the loops were plotted on a linear scale of 0 to 1, with 1.0 representing 15 kilobases of DNA. Ovomucoid structural sequences are represented by $\square$, intervening sequences by $\blacksquare$, and flanking DNA sequences by —.

and position can be correlated with known distances in the structural sequence. However, in the absence of favourably placed restriction sites, some intervening sequences may be missed. Electron microscopy permits more direct analysis of intervening sequences; in our studies, it has detected two additional intervening sequences, but the overall structure was in good qualitative agreement with the limited restriction map.

There are at least six intervening sequences in the ovomucoid gene. This is, necessarily, a minimum number; nucleotide sequence data are required for a complete structural charac-

terisation. A short structural sequence at the 5'-end may not form a stable hybrid in the conditions used. A 5.5-kilobase potential RNA precursor has been detected in nuclei from stimulated oviducts (see accompanying paper²⁰). Preliminary RNA-excess hybridisation experiments with fragments of the cloned gene have indicated that an additional 1.5 kilobases of DNA to the left of the unique *Kpn*I site may be transcribed *in vivo*. These observations suggest that the difference in size between the gene (Fig. 4) and the high molecular weight RNA transcript may be due to an undetected intervening sequence at the 5'-end. The size of the ovomucoid gene then, including the structural sequence beyond the *Eco*RI site, is about 5.5 kilobases, which is in good agreement with the estimated size of the largest RNA precursor. As the *Eco*RI site in the structural gene is a few nucleotide pairs to the right of the termination codon, all six intervening sequences observed in OM15 DNA are situated within the peptide-coding portion of the ovomucoid gene.

Although intervening sequences have been observed in several other cloned eukaryotic genes^{10-14,21-29}, the frequency of occurrence of these sequences in the ovomucoid gene is striking. Even in the absence of additional intervening sequences in the 3'-untranslated region, there is a minimum of six such sequences within a structural gene only about 750 nucleotide pairs long. At 1 intervening sequence per 125 nucleotide pairs of structural gene sequence, it represents the highest frequency of intervening sequences observed among all eukaryotic genes examined to date.

With regard to the expression of the ovomucoid gene *in vivo*, the entire gene is apparently transcribed into a large RNA transcript which is subsequently spliced to yield the mature mRNA. The identification of high molecular weight ovomucoid RNA precursors by methylmercury hydroxide agarose gel electrophoresis followed by transfer of RNA to diazobenzyloxymethyl paper and hybridisation with labelled pOM100 is presented in the accompanying paper²⁰. In this regard, the ovomucoid gene is similar to other genes with intervening sequences, such as those coding for chick ovalbumin³⁰, mouse globins³¹, mRNAs of animal viruses^{32,33} and yeast tRNAs^{34,35}. Finally, studies of the structures and the expression of the ovalbumin and ovomucoid genes should further our understanding of the regulation of these chick oviduct genes.

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Note added in proof: Further electronmicroscopic mapping of the gene has indicated that there is a seventh intervening sequence. A short structural gene segment has been detected in intervening sequence A.

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Identification of potential ovomucoid mRNA precursors in chick oviduct nuclei

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Denaturing gel electrophoresis of chick oviduct nuclear RNA reveals multiple species of RNA that are 1.5–5 times larger than ovomucoid mRNA. By analogy with ovalbumin RNA processing, these data suggest that ovomucoid mRNA is derived from a primary transcript that contains intervening sequences.

ADMINISTRATION of oestrogen to immature chicks induces the accumulation of the mRNAs for the egg-white proteins ovalbumin^{1–7}, ovomucoid^{8,9}, conalbumin¹⁰ and lysozyme⁸ in oviduct target cells. Of these four mRNAs, that of ovalbumin is the most abundant and has therefore been most extensively studied. Several lines of evidence^{7,11,12} suggest that ovalbumin mRNA (mRNA_{ov}) is derived from a large nuclear precursor RNA which is a transcript of the chick ovalbumin gene which is interrupted by at least seven regions of intervening sequence DNA^{13,14}. RNA sequences complementary to the intervening sequence regions were detected in oviduct nuclei⁷, but not in oviduct polysomes¹¹, indicating that they are not transported from the nucleus. In addition, multiple species of nuclear RNA larger than ovalbumin mRNA have been fractionated by gel electrophoresis and have been shown to contain both ovalbumin structural and intervening sequences⁷. We report here that there are also multiple species of nuclear RNA larger than the mRNA for ovomucoid. The experiments have been made possible because we have recently cloned complementary DNA (cDNA) to ovomucoid mRNA (mRNA_{om}) and prepared a DNA fragment that contains the cDNA sequence¹⁵. Using this specific probe, we have analysed the distribution of ovomucoid mRNA sequences in oviduct nuclear RNA that has been fractionated by agarose gel electrophoresis in completely denaturing conditions.

Identification of multiple nuclear species of ovomucoid sequence-containing RNA

To search for species of ovomucoid RNA larger than mature mRNA_{om}, nuclear RNA from oviducts of oestrogen-stimulated chicks was subjected to agarose gel electrophoresis in the presence of the denaturing agent, methylmercury hydroxide. Following transfer of the RNA from the gel onto diazobenzyloxymethyl (DBM) paper and hybridisation to a ³²P-labelled ovomucoid cDNA probe, multiple discrete bands containing ovomucoid structural sequences were observed (Fig. 1, lane A). The band with the greatest mobility co-migrated with mature mRNA_{om} (Fig. 1, lane B). Five major bands were significantly larger than ovomucoid mRNA (1,100 nucleotides). In addition, two minor bands were detected in certain nuclear RNA preparations (Fig. 2, lane C). As shown in Table 1, the sizes of the seven species of high molecular weight (MW) ovomucoid RNA, which were labelled a–g, ranged from 1.5 to 5 times the size of ovomucoid mRNA. The mobilities of the prominent ovomucoid RNAs (bands a, b, d, e, g) were reproducibly observed in all preparations of oviduct nuclear RNA that were analysed. Based on its intensity, band g, which has 1,700 nucleotides, seems to be the most abundant species of high MW ovomucoid RNA. Based on the radioactivity associated with each band, as determined by liquid scintillation counting, we estimate that 20–45% of the nuclear ovomucoid sequences reside in the forms higher in MW than mRNA_{om}.

High MW species of RNA that contain ovomucoid sequences were not found in oviduct cytoplasm, where only mature mRNA_{om} was detected (Fig. 1, lane C). In addition, the presence of nuclear high MW ovomucoid RNA, as well as that of mature mRNA_{om}, was tissue specific. No ovomucoid bands were detected in the nuclear RNA from spleen or liver that was isolated from oestrogen-stimulated chicks (Fig. 1, lanes D, E). Thus, high MW ovomucoid RNA, as well as mRNA_{om}, is present in significant amounts only in those tissues that actively synthesise egg-white proteins.

Although the conditions used for electrophoresis were rigorously denaturing^{7,18}, an attempt was made to generate high MW species of ovomucoid RNA by mixing liver nuclei and oviduct cytoplasm (both prepared from chicks stimulated with diethylstilboestrol (DES)) and then extracting the RNA from the mixture. However, mRNA_{om} was the only species detected (Fig. 1, lane F). Thus, as was observed previously for ovalbumin RNA⁷, the high MW species of ovomucoid RNA do not seem to be due to aggregation of mRNA_{om} with itself or other RNA molecules.

Comparison of the nuclear species of ovalbumin and ovomucoid RNA

To permit an exact comparison of the bands of high MW ovalbumin and ovomucoid RNA, oviduct nuclear RNA that had been electrophoresed and transferred to DBM paper was first hybridised to a ³²P-labelled probe specific for ovalbumin RNA sequences (Fig. 2, lane A). Following autoradiography, the ³²P-ovalbumin probe was eluted (Fig. 2, lane B) and the same DBM paper rehybridised, except this time to the ³²P-ovomucoid probe (Fig. 2, lane C). Two of the species of ovalbumin RNA (Fig. 2a,b) had significantly higher MWs than the largest species of ovomucoid RNA. Of all the bands observed, only ovalbumin RNA f and ovomucoid RNA b shared the same electrophoretic mobility. As the intensity of the bands differed, however, it is unlikely that they represent the same species of RNA. Thus, ovalbumin and ovomucoid RNA do not seem to have any of the high MW species in common.

Table 1 Sizes of the ovomucoid sequence-containing RNA from chick oviduct nuclei

RNA _{om} species	Relative mobility (to mRNA _{om})	Length (nucleotides)
a	0.25	5450 ± 120
b	0.45	3100 ± 60
c	0.48	2750 ± 60
d	0.55	2500 ± 60
e	0.59	2300 ± 50
f	0.65	2000 ± 30
g	0.76	1700 ± 75
mRNA _{om}	1.0	1100 ± 110

Relative mobility was calculated as the average of 6 individual experiments. Length was calculated by comparison with the electrophoretic mobility of RNA and DNA standards. RNA standards were chicken 27S rRNA (4,530 nucleotides), chicken 18S rRNA (2,076 nucleotides), *Escherichia coli* 23S rRNA (3,129 nucleotides) and *E. coli* 16S rRNA (1,550 nucleotides). DNA standards were obtained by digestion of λ DNA with *EcoRI* (21,746, 7,524, 5,920, 5,523, 4,793 and 3,380 nucleotides) or with *HindIII* (23,222, 9,730, 6,460, 4,492, 2,301, 2,000 and 682 nucleotides). In the conditions of electrophoresis (in the presence of methylmercury hydroxide), DNA becomes single stranded and its mobility is similar to that of RNA. Error limits indicate the standard deviation.

Based on the relative intensities of the bands, the concentration of ovalbumin mRNA, as expected, is greater than that of ovomucoid mRNA. By contrast, the high MW species of ovomucoid RNA are apparently more abundant in oviduct nuclei than the high MW species of ovalbumin RNA. It is of further interest that a significant portion of the nuclear ovalbumin RNA sequences was smaller than mRNA_{om}. By contrast, no significant degradation of mRNA_{om} was observed.

Identification of ovomucoid intervening sequences in high MW nuclear RNA

Restriction endonuclease mapping and electron microscopic analysis of a cloned DNA fragment reveal that, like the ovalbumin gene, the natural chick ovomucoid gene has at least six regions of intervening sequence¹⁹. Using as a hybridisation probe a clone with a DNA insert that seems to contain all the ovomucoid structural and intervening sequences, it has been possible to determine that the nuclear ovomucoid RNA molecules larger than the mature mRNA_{om} contain sequences complementary to the intervening sequences of the ovomucoid gene. Apart from band *f*, all the bands of ovomucoid nuclear RNA, including mature mRNA_{om}, that hybridised to the ovomucoid cDNA probe were again detected when hybridised to the natural ovomucoid gene probe (Fig. 3, lane A). In addition, two bands (located between bands *a* and *b*) that were extremely faint after hybridisation to the ovomucoid cDNA probe were clearly detected by the natural ovomucoid gene probe. To detect only those bands that contained intervening sequences, it was necessary to hybridise in the presence of excess unlabelled ovomucoid mRNA to compete out the structural sequences in the natural gene. In these hybridisation conditions, detection of the band corresponding to mature mRNA_{om} was eliminated (Fig. 3, lane B). In contrast, all the bands of high MW ovomucoid RNA were still detected. This indicates that all the species of ovomucoid RNA larger than mature mRNA_{om} contain sequences complementary to the intervening sequences of the ovomucoid gene.

Oestrogen-induced accumulation of high MW species of ovomucoid RNA

The accumulation in the oviduct nucleus of species of ovomucoid RNA larger than mature mRNA_{om} was dependent on oestrogen. From 20 µg of oviduct nuclear RNA isolated from chicks that had been withdrawn from DES treatment for 14 d, neither high MW ovomucoid RNA nor mRNA_{om} was detected (Fig. 4, left, lane W). Thus, in agreement with our previous studies using *R*₀t analysis⁹, the amount of ovomucoid RNA sequences in withdrawn oviduct nuclei is very low. When 100 µg of withdrawn oviduct nuclear RNA was analysed, a faint band of mRNA_{om} was observed (Fig. 4, right, lane W). Thus, the few ovomucoid RNA sequences that remain after hormone withdrawal seem to be present mainly in the form of mature mRNA_{om}.

As soon as 1 h after administration of DES to hormone-withdrawn chicks, both mature mRNA_{om} and high MW ovomucoid RNA species have accumulated. Analysis of 20 µg of nuclear RNA revealed the accumulation of the most abundant high MW species, band *g* (Fig. 4, left), whereas analysis of 100 µg of RNA revealed the accumulation of the three most predominant species larger than mRNA_{om}, bands *b*, *e* and *g*, (Fig. 3, right). Species *a*, *c*, *d* and *f* were not detected, probably because these are the least abundant of the high MW ovomucoid RNA molecules.

After short periods (1–2 h) of secondary oestrogen stimulation, the relative intensity of the ovomucoid RNA bands (Fig. 4, right) was similar to that observed after chronic oestrogen stimulation (Fig. 1, lane A; Fig. 2, lane C). The bands cor-

responding to species *g* and mRNA_{om} were of roughly equal intensity, whereas that of species *e* was of lesser intensity and that of species *b* even less. After longer periods (>4 h) of secondary stimulation, this pattern shifted. The concentration of mRNA_{om} increased at each time point so that 16 h after the DES injection a very intense mRNA_{om} band was observed. By contrast, the concentration of the most abundant high MW ovomucoid RNA species, band *g*, increased only slightly during this period. In addition, no significant increases in the concentration of ovomucoid species *e* were observed. Thus, 4–16 h after DES injection, mature mRNA_{om} was clearly more abundant than any of the high MW ovomucoid RNA species.

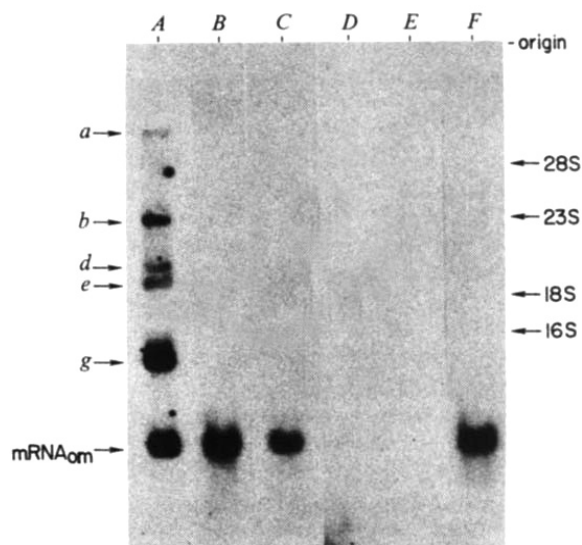


Fig. 1 Identification of ovomucoid mRNA sequences in high MW nuclear RNA. Nuclear or cytoplasmic RNA was isolated from the indicated tissues from White Leghorn chicks stimulated chronically with diethylstilboestrol (DES) as described previously⁷. Ovomucoid mRNA (~40% pure) was prepared from hen oviduct as previously described¹⁵. RNA was then subjected to agarose gel electrophoresis in the presence of 10 mM methylmercury hydroxide (Alfa) as described previously⁷. All procedures involving methylmercury hydroxide were carried out in a fume hood and all waste was disposed of as for hazardous chemicals. The RNA was transferred by blotting from the gel to diazobenzylxymethyl (DBM) paper by the method of Alwine *et al.*¹⁶. Following transfer the DBM paper (12 × 14 cm) was hybridised to the ³²P-labelled ovomucoid probe (3 × 10⁷ c.p.m., specific activity 5 × 10⁸ c.p.m. per µg) in the conditions used previously⁷. Following hybridisation, the DBM paper was washed 3 times in 200 ml of 0.5 × SSC (75 mM NaCl, 7.5 mM Na citrate, pH 7.0) for 2 h at 65 °C. This washing procedure significantly reduced the background during autoradiography. The DBM paper was then dried and autoradiographed for 16 h using a Dupont Cronex intensifying screen at -20 °C. The probe specific for ovomucoid RNA sequences was prepared from a *Hha*I fragment of pOM100, a chimaeric plasmid which was previously constructed by our laboratory and contains an ovomucoid cDNA insert¹⁵. This fragment, which contained ~700 base pairs that correspond to the sequence of ovomucoid mRNA, was labelled by nick translation by a modification of the procedure used previously⁷. The reaction was carried out in a final volume of 10 µl which contained: 50 mM Tris, pH 7.8; 5 mM MgCl₂; 10 mM 2-mercaptoethanol; 0.5 µg of bovine serum albumin; 60 µM dATP, dGTP; 6 µM unlabelled dCTP, dTTP; 3 µM ³²P-dCTP, dTTP (2,000 Ci mmol⁻¹ each, Amersham) and 0.1 µg of DNA. These components were assembled on ice and then 28 pg of DNase (Worthington, DPFF) was added. The mixture was incubated at room temperature for 1 min and immediately cooled in an ice water bath. *E. coli* DNA polymerase (2 µl, 9 units, Boehringer Mannheim) was added and the mixture incubated at 14 °C for 4 h. The reaction was stopped by the addition of 25 µl of 40 mM EDTA, 25 µl of *E. coli* DNA (4 mg ml⁻¹) and 3 µl of 20% SDS. Unincorporated dNTPs were separated from the labelled DNA by gel filtration on Sephadex G-50 in 10 mM Tris-HCl, pH 7.5, 100 mM NaCl. Radioactive fractions eluting in the void volume were pooled, made 0.2 M in Na acetate, pH 4.5, and precipitated by addition of two volumes of ethanol. The specific activity of the ³²P-DNA was 5–10 × 10⁸ c.p.m. per µg. A, Oviduct nuclear RNA, 20 µg. (High MW ovomucoid RNA species *c* and *f* were not detected in this preparation.) B, Ovumucoid mRNA purified to approximately 40%, 15 ng. C, Oviduct cytoplasmic RNA, 20 µg. D, Spleen nuclear RNA, 20 µg. E, Liver nuclear RNA, 20 µg. F, RNA isolated after mixing liver nuclei with oviduct cytoplasm, 20 µg. The position of 28S, 23S, 18S and 16S ribosomal RNA markers is indicated.

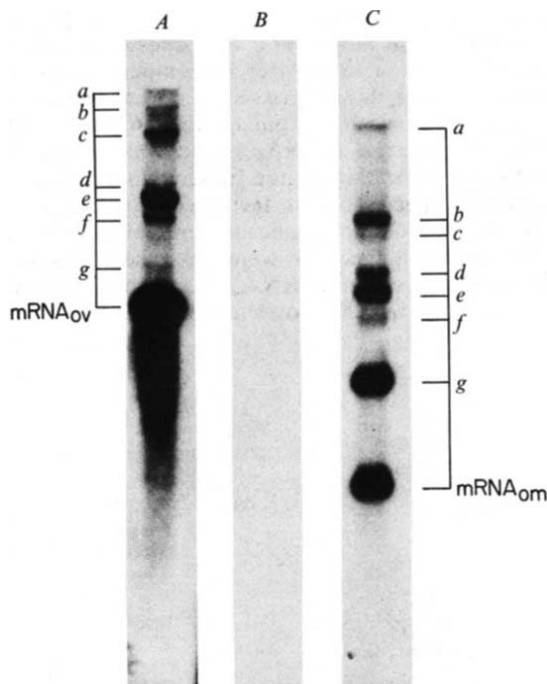


Fig. 2 Comparison of ovomucoid and ovalbumin nuclear RNA. Oviduct nuclear RNA from chronically DES-stimulated chicks, 20 μ g, was electrophoresed and transferred to DBM paper (2 $\times$ 14 cm), hybridised to the 32 P-ovalbumin probe (5×10^6 c.p.m., specific activity 5×10^8 c.p.m. per μ g) and autoradiographed (lane A). The probe specific for ovalbumin sequences was prepared from a *Hha*I fragment of a nearly full-length ovalbumin cDNA clone, pOV230, as described previously⁷. Following autoradiography, the 32 P-ovalbumin probe that was hybridised to the RNA bound to the DBM paper was eluted by incubation in 200 ml of 99% formamide, 0.1 $\times$ SSC (15 mM NaCl, 1.5 mM Na citrate, pH 7.0) for 45 min at 65 $^{\circ}$ C. The paper was then washed twice in 200 ml of water 2 h at 65 $^{\circ}$ C. The completeness of the elution was checked by autoradiography (lane B). The DBM paper then hybridised to the 32 P-ovomucoid probe (5×10^6 c.p.m., specific activity 5×10^8 c.p.m. per μ g) and autoradiographed (lane C).

Comments

We have demonstrated the existence in chick oviduct of multiple species of ovomucoid RNA larger than mature mRNA_{om}. These high MW forms of ovomucoid RNA are found in oviduct nuclei, but not in spleen or liver nuclei, nor in oviduct cytoplasm. They do not result from aggregation of mRNA_{om}. The high MW forms contain sequences complementary to the intervening sequences as well as to the structural sequences of the ovomucoid gene. Their accumulation in oviduct nuclei is regulated by oestrogen. These results suggest that ovomucoid mRNA may be synthesised by the processing of a large primary gene transcript and that the multiple bands of ovomucoid RNA larger than mRNA_{om} may represent transcripts at different stages of processing.

To date, mRNA precursors have been carefully characterised only for β -globin mRNA. A 15S globin RNA molecule has been demonstrated to be a precursor to mRNA by pulse-label and chase experiments²⁰⁻²⁴. Electron microscopic analysis of globin RNA-DNA hybrids has shown that this 15S molecule consists of a continuous transcript of the three structural and two intervening sequence regions of the β -globin gene^{25,26}. In addition, a smaller precursor has been identified that probably contains only one of the intervening sequences of the globin gene^{22,23}. These results suggest that discrete species of RNA may exist for each combination of intervening sequences. Thus, the observation that the species of ovomucoid RNA larger than mRNA_{om} are comprised of both structural and intervening sequences of the ovomucoid gene strongly suggests that the high MW species represent mRNA_{om} precursors that lose intervening sequences as they are processed.

The kinetics of accumulation in oviduct nuclei of ovomucoid RNA sequences after secondary oestrogen stimulation is also consistent with the hypothesis that the high MW forms are precursors of mRNA_{om}. First, the prominent high MW species of ovomucoid RNA were detected after only 1 h of secondary stimulation. Second, at early times (1-2 h) after oestrogen administration, the concentration of species g seems to be the same as that of mRNA_{om}. After longer periods (up to 16 h), the concentration of species g increased only slightly, whereas that of mRNA_{om} increased to much higher levels. Although other explanations are possible, this differential accumulation is consistent with the hypothesis that the high MW species of ovomucoid RNA are efficiently processed to form mature mRNA_{om}. If they are precursors, this species should be detected before mRNA_{om}. Although this was not observed, it is possible that accumulation of high MW forms of ovomucoid RNA before that of mRNA_{om} might be observed if oviduct nuclear RNA is analysed within <1 h of DES injection. However, these detailed kinetic studies are difficult to control with intact animals and are perhaps better approached using an organ culture system where newly synthesised RNA may be radioactively labelled.

As the largest species of ovomucoid RNA is fivefold larger than mRNA_{om}, at least 80% of the ovomucoid gene may be intervening sequence DNA. In comparison, we estimate the ovalbumin gene to contain about four times more DNA than required to encode mRNA_{ov}. Thus, the overall organisation and structure of the ovomucoid gene is similar in complexity to that previously described for the ovalbumin gene^{11,12}. However, studies of the ovomucoid RNAs may offer advantages over those of the ovalbumin RNAs in the elucidation of precursor-product relationships. First, the fraction of nuclear ovomucoid RNA that is in the form of high MW species seems to be greater than that of nuclear ovalbumin RNA. Second, the ovomucoid

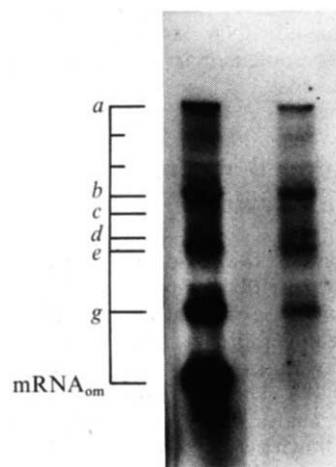


Fig. 3 Identification of ovomucoid intervening sequences in high MW ovomucoid nuclear RNA. For these experiments a hybridisation probe was initially prepared from a clone, selected from a library of chicken DNA fragments that were cloned in Charon 4A phage containing a 15-kilobase ovomucoid natural gene insert. This clone, which will be described in detail elsewhere²⁷, seems to contain all the structural and intervening sequences of the ovomucoid gene. Total DNA was isolated from the cloned phage, labelled with 32 P by nick translation and used as a hybridisation probe. Subsequent experiments in which the cloned ovomucoid fragment described in the accompanying paper¹⁹ was used as a hybridisation probe gave identical results. Oviduct nuclear RNA from chronically stimulated chicks, 20 μ g, was electrophoresed, transferred to DBM paper (2 $\times$ 14 cm) and allowed to hybridise to the 32 P-labelled natural ovomucoid gene probe (10^7 c.p.m., specific activity 6×10^8 c.p.m. per μ g) in the absence (lane A) or presence (lane B) of unlabelled ovomucoid mRNA as a competitor. The conditions for competition were as follows: ovomucoid mRNA (20 μ g, ~20% pure) was preincubated with the 32 P-labelled probe in 1.0 ml of hybridisation buffer at 42 $^{\circ}$ C for 1 h. The mixture was diluted to 5.0 ml with hybridisation buffer and then hybridised to the DBM paper containing the bound nuclear RNA. Autoradiography of both samples was for the same length of time (about 1 week).

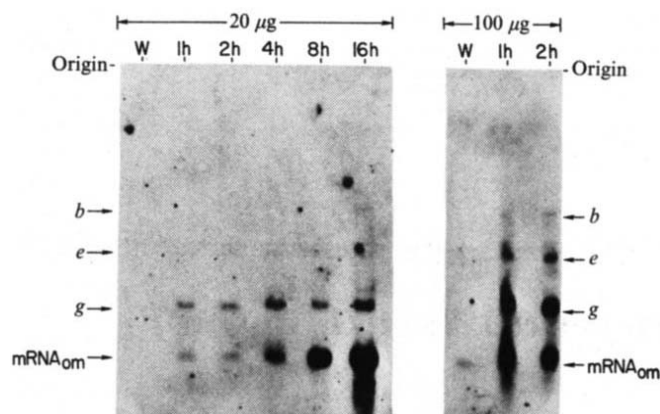


Fig. 4 Induction of high MW ovomucoid RNA by secondary oestrogen stimulation. Oviduct nuclear RNA was isolated from chicks that had received daily subcutaneous injections of 2.5 mg of DES for 14 d and subsequently withdrawn from hormone for 14 d (W). In addition, hormone-withdrawn chicks were injected with a single dose of 2.5 mg of DES and the oviduct nuclear RNA was isolated after 1, 2, 4, 8 and 16 h. The RNA was electrophoresed, transferred to DBM paper (15 × 15 cm) and hybridised to the ^{32}P -ovomucoid probe (3.3×10^7 c.p.m., specific activity 8×10^8 c.p.m. per μg). Left panel, 20 μg of nuclear RNA was analysed; right panel, 100 μg of nuclear RNA was analysed.

RNA bands are well separated from each other on the gel, whereas the ovalbumin RNA bands are more closely spaced. These two features should enhance the assessment of planned kinetic labelling studies as well as the purification of individual species of high MW nuclear RNA.

R_{ot} analysis has previously shown that the concentration of total ovomucoid RNA sequences in oviduct nuclei gradually increased from approximately 1 part per 10^6 to 30 parts per 10^6 after 16 h of secondary oestrogen stimulation⁹. These results are consistent with the accumulation of individual nuclear ovomucoid RNA species shown in Fig. 3. Both analyses clearly show that the low concentration of mRNA_{om} in withdrawn oviduct polysomes⁸ is not the result of nuclear sequestration of immature or mature forms of mRNA_{om} . In addition, by hybridising nuclear radiolabelled mRNA_{om} to an excess of cloned

ovomucoid DNA, we have been able to estimate the rate of mRNA_{om} synthesis and have shown it to depend on oestrogen⁹. Taken together, these results are consistent with regulation of the ovomucoid gene by oestrogen mainly through control of its transcription.

The library of chicken DNA fragments which were cloned in Charon 4A phage was provided by Tom Maniatis and Richard Axel. We thank Ms Melanie Vinion and Mr William Tate for technical assistance. This work was supported by NIH grant HD-8188, the Baylor Center for Population Research and Reproductive Biology. J.L.N. is the recipient of USPHS Postdoctoral Fellowship GM 06893-01. All cloned DNA was prepared using a P3 facility in accordance with the NIH guidelines.

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letters

A model for the unpulsed light from the Crab pulsar

THE recent detection¹ of a significant unpulsed fraction of the light from the Crab Nebula pulsar invites comment regarding models for the beaming of the radiation. One such model is the relativistic beaming model²⁻⁴ in which the radiation comes from a region near the speed-of-light cylinder of a neutron star magnetosphere. Here the predictions of this model are investigated, using model parameters found by Ferguson *et al.*⁵ from fits to the polarisation characteristics of the Crab pulsar, and which were subsequently found⁶ to agree with the change of light curve with colour of the secondary pulse.

The parameters are β , the fraction of the light cylinder radius at which the radiation is emitted; T , the angle between the rotation equator and the line of sight; and ϵ , the spectral index. It is assumed that in the frame of reference of the emission regions co-rotating with the neutron star the radiation is unbeamed, and

only becomes pulsed to an outside observer because of the Doppler effect and the aberration of light.

In the following, quantities in the corotating frame are primed, and those to an outside observer unprimed. Subscript 1 refers to the main pulse emission and 2 to the secondary pulse emission. Subscripts max and min refer to the maximum and minimum of the quantities observed by an outsider. In the relativistic beaming theory, the intensity

$$I = RI'$$

where $R = (1 - \beta \cos \theta \cos T)^{-3-\epsilon}$, θ is the angle in the emission region orbit between the emission region and the point of minimum radial velocity, and ϵ is the spectral index from $I \propto \nu^{-\epsilon}$. $\beta_1 = 0.65$, $\beta_2 = 0.50$, $T = 25^\circ$ (from ref. 5), and $\epsilon = 0.2$ (from ref. 7) Then,

$$I_{1,\text{peak}} \approx I_{1,\text{max}} + I_2(\theta_2) \quad (\theta_1 = 0)$$

$$I_{2,\text{peak}} \approx I_{2,\text{max}} + I_1(\theta_1) \quad (\theta_2 = 0)$$

and $I_{\min} \approx I_2(\theta_{2m}) + I_1(\theta_{1m})$

Here, $I_{\min}$ is the minimum of the observed intensity, taken to occur midway between the secondary and main pulses, θ_{1m} is the value for emission region 1 at that time, and θ_{2m} is the corresponding value for emission region 2. θ_1 is the value of θ for emission region 1 at the time of the secondary pulse peak and θ_2 is the value for emission region 2 at the time of the main pulse peak.

To compute the values of θ , we must relate the observed pulse longitudes to phases in the emission region orbits through the relationship⁸

$$\theta_{\text{obs}} = (\theta - \beta \sin \theta \cos T)$$

Taking the separation of the main and secondary pulses to be 146° , we have

$$146^\circ = -(\theta_2 - \beta_2 \sin \theta_2 \cos T) = (\theta_1 - \beta_1 \sin \theta_1 \cos T)$$

and at the midpoint of their greatest separation,

$$\begin{aligned} \frac{(360 - 146^\circ)}{2} &= (\theta_{2m} - \beta_2 \sin \theta_{2m} \cos T) \\ &= -(\theta_{1m} - \beta_1 \sin \theta_{1m} \cos T) \end{aligned}$$

Solving for the various values of θ and substituting, we find

$$I_{1,\text{peak}} \approx 17.22I'_1 + 0.329I'_2,$$

$$I_{2,\text{peak}} \approx 6.90I'_2 + 0.247I'_1$$

and

$$I_{\min} \approx 0.345I'_1 + 0.458I'_2$$

Now from the tabulated light curves with $I_{\min}$ subtracted⁶ we find

$$(I_{2,\text{peak}} - I_{\min}) = 0.270(I_{1,\text{peak}} - I_{\min})$$

Solving this for I'_2 , we find $I'_2 = 0.719I'_1$, which shows that the intensities of both pulses are roughly equal in their emission frames. Finally, on substituting we have $I_{\min}/I_{1,\text{peak}} \approx 0.039$, or the minimum in observed intensity should be $\sim 3.9\%$ of the main pulse peak.

The observed values of Peterson *et al.*¹ are $3.6\% \pm 0.7$ and $3.7\% \pm 0.8$, using two independent techniques. The agreement between the theoretical and observed values is remarkable. The simplistic model predicts an underlying component of emission almost identical to that observed, and does this with no free parameters. Of course, to explain the Crab pulsar's asymmetrical pulse shapes, Cocke and Ferguson⁶ postulated a certain amount of intrinsic beaming to supplement the relativistic beaming. Also, the recent resolution of the Crab pulsar optical main peak⁹ indicates a width consistent with $\beta \approx 0.9$. If this pulse width is indicative of the true value of β for the main peak, the unpulsed light would need to come mainly from the secondary pulse emission region, which has a much lower value of β . It is not clear whether the inclusion of these effects would worsen the numerical agreement arrived at by the above analysis. However, the fact that this simple version of the model agrees even roughly with the observations, using the same model parameters necessary to fit the polarisation and change of light curve with colour, is further evidence of the power of the relativistic beaming model for the Crab Nebula pulsar.

With the new information about the unpulsed emission, it would be worthwhile to reanalyse the old Crab polarisation data in the hope of correcting the percentages and angles near the polarisation minima, which are most affected by errors in the subtracted noise level, and to see how the polarisation behaves in the regions between pulses. If the model given here is correct, the between-pulse polarisation should be high and smoothly vary from one emission region to the next.

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Ion-induced dipole H_n^+ clusters

THERE has long been an interest in the study of ion-induced dipole clusters of the type H_n^+ (n , odd integer). These positive clusters may be present in gaseous nebulae^{1,2}, and Clappitt and Gowland³ have discovered in mass spectrometer experiments such H_n^+ ions for $3 \leq n(\text{odd}) \leq 99$. The ions are very weakly bound because of the nature of the ion-induced dipole forces involved, and thus must be produced at low temperatures. In these experiments a layer of hydrogen gas is deposited on a metal surface maintained at 3 K in a baked vacuum apparatus operating at $\sim 10^{-10}$ torr. The surface is swept by a 23 eV electron beam and the ions thus created are focused into a quadrupole filter and detected. The surface mechanism for production of the ions is not understood but the presence of grains in the interstellar medium might provide surfaces which could play an analogous role in similar conditions of low pressure and temperature. To study the binding energy and geometry of such clusters we performed *ab initio* Hartree-Fock-Roothaan calculations⁴, using a contracted s-type Gaussian orbital basis. Our previous use of this type of calculation⁴⁻⁷ for ion molecule complexes which dissociate into closed shell fragments, indicated that reliable results can be obtained. The results shown in Fig. 1 and Table 1 are all based on this type of calculation implemented with the Gaussian 70 program^{8,9} using four gaussians per hydrogenic s-orbital. As expected, we found the positive cluster to be quite weakly bound, that is the binding energy was of the order of 1 kcal mol⁻¹ against dissociation according to



(the largest and smallest binding energies found were 6.0 kcal mol⁻¹ for H_3^+ and 0.3 kcal mol⁻¹ for H_{15}^+). In our calculations the geometries of the positive clusters were dominated by the strongly bound triangular nucleating centre H_3^+ . Each member of the series H_n^+ , $5 \leq n(\text{odd}) \leq 15$ is formed by the addition of one hydrogen molecule to the basic structure of the previous member in the series. We report here calculated binding energies and geometries for the negative ion induced dipole clusters H_n^- , $5 \leq n(\text{odd}) \leq 13$.

These ions have not apparently been previously investigated theoretically or experimentally although a search for such clusters was suggested by Clappitt and Gowland³. It would be interesting to investigate whether the binding energies are of the same order of magnitude as in the analogue clusters H_n^+ , as it has been established that in this case the clusters may be experimentally produced in favourable conditions². As in the case of

Table 1 Calculated binding energies and geometrical parameters for hydrogen clusters H_n^-

Molecule	Binding energy (kcal mol)	Geometric parameters		
		<i>a</i> (Å)	<i>b</i> (Å)	<i>c</i> (Å)
H_3^-	8.91	2.05	0.74	
H_5^-	7.30	2.10	0.74	
H_7^-	6.52	2.12	0.74	
H_9^-	5.59	2.15	0.74	
H_{11}^-	4.00	2.21	0.74	2.27
H_{13}^-	3.97	2.34	0.74	

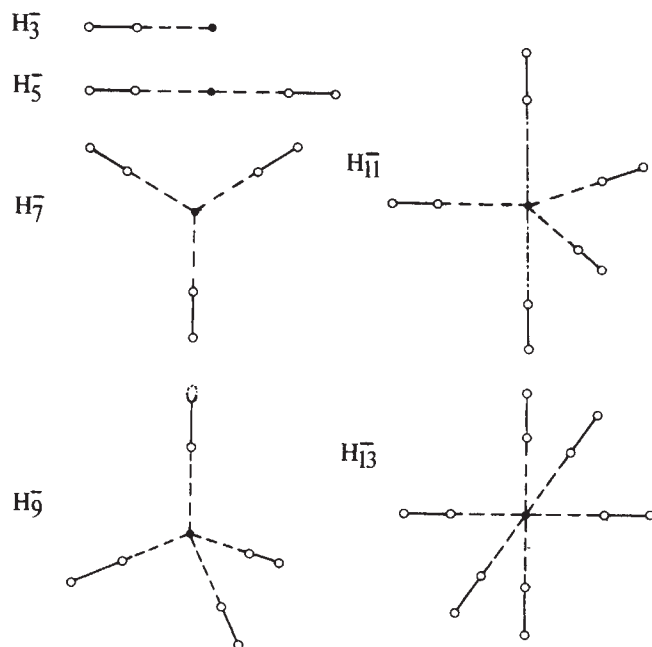


Fig. 1 Geometry of H_n^- clusters. See Table 1 for magnitudes of bond distances: a, —; b, ---; c, - · - ·.

the positive clusters⁴, the conditions in gaseous nebulae may be suitable for weakly bound negative clusters to exist (some of the conditions that have been laid down¹⁰ for a possible molecular source of the diffuse interstellar absorption lines are in fact met by the H_n^- and H_n^+). We expected that the geometries for the negative ions would be quite different than for the positive ions as both the charge sign and the geometry of the nucleating centre differ. In the latter, the tightly bound triangular geometry of the nucleating centre H_3^+ governs the geometries of the clusters of increasing mass. It is energetically favourable for a positive charge to be approached by a hydrogen molecule along a line perpendicular to its bond. In the former, the nucleating centre is a H^- ion which presents an essentially spherical static electric field to H_2 molecules in the clusters. It is energetically favourable for a negative charge to be approached by a hydrogen molecule along a line through its bond. The molecular ion H_3^- dissociates in ordinary conditions according to the reaction



although it^{11,12} exhibits a shallow potential minimum (about $0.8 \text{ kcal mol}^{-1}$ for a linear geometry with H^- about $2.41 \text{ \AA}$ to the nearest atom in H_2) capable of supporting discrete vibrational and rotational states in favourable experimental conditions. Our calculations also reproduce the shallow potential minimum for H_3^- in a linear geometry, found by previous workers. At the geometry of minimum energy we find the nucleating centre H^- at a distance of $2.05 \text{ \AA}$ from the nearest atom in the H_2 fragment. The H_2 fragment has a bond distance equal to $0.74 \text{ \AA}$, the same as for the free H_2 molecule.

Our new results are summarised in Fig. 1 and Table 1. All these results correspond to calculations in which the total energy of the system has been optimised with respect to all geometrical parameters (bond lengths and bond angles) within the system.

For each molecule the energy surface displayed a potential well whose minimum was sufficiently deep enough to support a few discrete vibrational and rotational states and which geometrically correspond to ion-induced dipole bonds. All the clusters contain polarised H_2 fragments (having a bond distance corresponding to the free H_2 molecule) attached to the negative ion nucleating centre H^- . In fact the geometries adopted by the various clusters under energy optimisation correspond to Gillespie's rules^{13,14} of molecular geometry based on minimisation of repulsion of electron pairs.

Table 1 shows that all of the clusters displayed are bound by a few kilocalories per mole against the dissociation



a result which is analogous to the previous calculation for the experimentally known H_n^+ molecular ions.

The binding energy of the H_3^- ion is $7.3 \text{ kcal mol}^{-1}$, and has a linear geometry ($C_{\infty v}$) with the nucleating centre $2.10 \text{ \AA}$ from either H_2 fragment. H_7^- has a binding energy of $6.52 \text{ kcal mol}^{-1}$. The geometry of the ion is planar and trigonal (D_{3h}) with an H_2 fragment to nucleating centre distance of $2.12 \text{ \AA}$. H_5^- is bound by $5.59 \text{ kcal mol}^{-1}$ with a tetrahedral geometry (T_d) and fragment to centre distance $2.15 \text{ \AA}$. The ion H_{11}^- decreases in bonding energy to $4.00 \text{ kcal mol}^{-1}$ and has a geometry which is trigonal bipyramidal (D_{3h}) with fragment to centre distances $2.21 \text{ \AA}$ and $2.27 \text{ \AA}$ as shown in Fig. 1. Finally H_{13}^- is found in its minimum energy configuration to be bound by $3.97 \text{ kcal mol}^{-1}$ in an octahedral geometry (O_h) with fragment to centre distance $2.34 \text{ \AA}$. This last result answers the question raised by Clampitt and Gowland³ on whether H_{13}^- might be the most stable of the negative clusters. We find that it is not. In fact Table 1 shows that for the negative ion clusters studied there is a continual decrease of binding energy as the size of the cluster grows.

In conclusion our results indicate that the negative ion clusters H_n^- (n is odd) are bound by a few kilocalories per mole, the same order of magnitude as found earlier for binding energy of the positive ion clusters H_n^+ (n is odd) which are experimentally known. The negative ions may form in favourable conditions.

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Polonium haloes in mica

GENTRY¹ has reported that some anomalous haloes found in ancient mica seem to arise from the α -decay of polonium isotopes only, without any observable long-lived β -emitting precursors. Fremlin² suggested that these might occur as a result of the diffusion of β -emitting radiogenic lead isotopes from relatively distant or widely dispersed uranium or thorium inclusions, and the absorption of the lead ions by small pre-existing inclusions of foreign material. As Gentry³ pointed out,

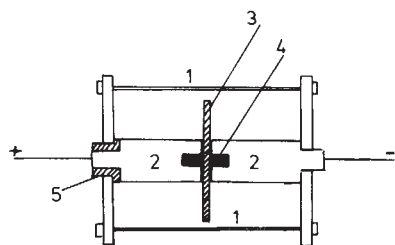


Fig. 1 Apparatus used to introduce lead into mica at elevated temperatures and under an applied electrical field. The muscovite mica sheet ($\sim 3 \times 2.4 \text{ cm}^2$, $200 \mu\text{m}$ thick) is held tightly, with the help of screws, between two stainless-steel cylinders (diameter 8 mm, height 2 cm). Central holes (diameter 3 mm, depth 6 mm) in these cylinders are filled with lead. The electrical field ($\sim 10^5 \text{ V cm}^{-1}$) is applied across the sample through insulated wires, and the whole assembly is placed inside a furnace. 1, Screw; 2, cylinder; 3, mica; 4, lead; 5, insulator.

this would involve abnormally high diffusion rates for lead. We have carried out experiments that demonstrate the possibility of such high rates. Here we also discuss the reasons for the absence of an excess of observable tracks left in mica by the recoil of the lead daughter when α -decay takes place in the polonium isotopes.

The method used in this work involved two major techniques: (1) introducing lead into mica and establishing conditions for diffusion; and (2) radioactivating to transform some of the lead nuclei to radioactive products, and the detection of the radioactivities by autoradiography using plastic nuclear track detectors. These two stages are considered below.

To study the diffusion of lead in mica, we first need to introduce an adequate number of lead atoms into the samples. This has been done by using the apparatus shown in Fig. 1. This

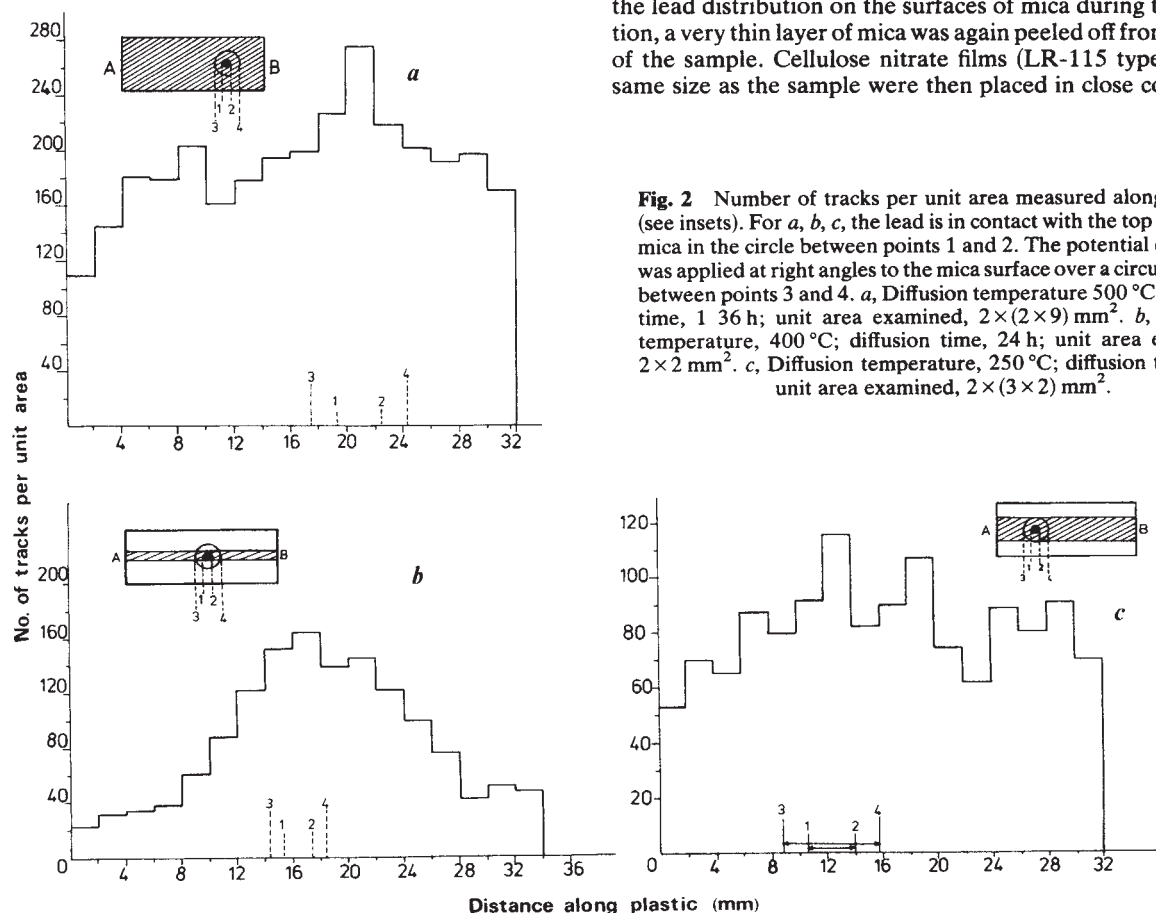
apparatus comprises two identical steel cylinders of diameter 8 mm and height 2 cm. Each cylinder has a coaxial hole of diameter 3 mm and depth 6 mm. These holes were filled with lead, and the mica sample ($\sim 3 \times 2.4 \text{ cm}^2$ in area) was clamped between the cylinders by the screws shown. The cylinders were connected to a power supply by heat-resistant insulated wires. The apparatus was then placed in a furnace at the desired temperature for periods of up to 48 h.

An electrical field ($\sim 10^5 \text{ V cm}^{-1}$) was applied at right angles to the cleavage planes of the mica. Any field-induced diffusion of lead was parallel to the field and between the areas in contact with lead (within a circle of diameter 3 mm), or at most between the areas over which the potential difference was applied (within a circle of diameter 8 mm). Any diffusion outside this region would be independent of the applied electric field.

A variety of activation methods have previously been used for lead analysis. For determination of the spatial distribution of lead in mica the reactions producing γ and/or β emitters are not suitable, as the interference from other γ and/or β emitters produced in the matrix is always very high.

We used ^3He and ^4He bombardments to produce α -particle-emitting isotopes of polonium according to reactions of the type $\text{Pb}(\text{He}, n)\text{Po}$. α -particles from the polonium could then be detected using cellulose nitrate films (Kodak LR-115, type II; Kodak-Pathé).

Rectangular pieces of muscovite mica ($\sim 3 \times 2.4 \text{ cm}^2$) of thickness $\sim 200 \mu\text{m}$ were placed between the two cylinders of the apparatus shown in Fig. 1. The assembly was placed in a furnace at a desired temperature and an electrical field of $\sim 10^5 \text{ V cm}^{-1}$ was applied between the cylinders. After several hours of diffusion (across and along the cleavage planes of mica sample), the sample was removed from the apparatus and a few layers of mica were peeled away from each face of the sample to remove any surface contamination. The remaining part of the sample was irradiated with ^3He or ^4He ions, of energy 30 MeV or 40 MeV respectively, and with a current of $2 \mu\text{A}$ for a period of 2 h on each face. To avoid the possibility of the disturbance of the lead distribution on the surfaces of mica during the irradiation, a very thin layer of mica was again peeled off from each face of the sample. Cellulose nitrate films (LR-115 type II) of the same size as the sample were then placed in close contact with



each surface of the sample. After several days of exposure, the plastics were etched in a 25% solution of sodium hydroxide at a temperature of 55 °C for 2 h. The resulting α -tracks were counted under an optical microscope, using an overall magnification of $\times 40$.

This diffusion experiment was carried out at temperatures of 600, 500, 400 and 250 °C for different lengths of time. Figure 2a, b, c shows some of the results.

Although none of the results shown in Fig. 2a, b, c can provide an accurate numerical value for the migration velocity of lead atoms in mica, they show that diffusion of lead in mica is a fairly rapid process and that the traversal of at least 1 cm was possible in 24 h over a considerable range of temperatures.

It may be true, as assumed by Gentry³, that the diffusion coefficient of lead is only of the order of $10^{-18} \text{ cm}^2 \text{ s}^{-1}$ at 20 °C in the case of field-free diffusion across the cleavage plane. Our observations suggest that, even at 500 °C and with an applied field of 10^5 V cm^{-1} , the diffusion coefficient D across the planes is $< 6 \times 10^{-10} \text{ cm}^2 \text{ s}^{-1}$. But Fig. 2a, b, c indicates that, at elevated temperatures, it must be much higher along the cleavage planes than the above-mentioned value: for example, $D > 3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ at 250 °C in the field-free area.

Gentry³ has pointed out that if active lead or polonium isotopes do diffuse through mica, recoil tracks should be observable from nuclei, which had undergone α decay during their diffusion through the mica. Such tracks should have appeared as 'excess' α -recoil tracks because during diffusion, each of the daughters recoiling from its α decay would have produced a separate track, whereas in the case of stationary U , Th nuclei the daughters of which did not diffuse away, the whole decay chain would have looked like a single, local recoil. No such excess recoil tracks have, in fact, been observed. We explain this apparent anomaly as follows.

Recoil tracks produced by α -decaying nuclei (α -RT) have an etchable range in mica of $\sim 0.01 \mu\text{m}$ (a combined fission-fragment etchable range of $\sim 20 \mu\text{m}$). The much shorter α -recoil tracks would fade more easily than the longer, more energetic, fission tracks, when subjected to thermal annealing in nature (see refs 4, 5; this conclusion is also supported by our unpublished data). The work of Nagpaul *et al.*⁶ confirms this fact.

They have shown that, in a biotite sample (from the Nellore mica belt of India), the length reduction of fossil fission tracks due to natural annealing at normal temperatures ($\sim 20^\circ\text{C}$) was $\sim 12\%$, which corresponds to 6.5% reduction in the apparent fossil-fission track density. This phase had been preceded by a high-temperature period lasting for about 190 Myr, during which the average temperature of the region must have been about 150 °C. This earlier period of geological annealing at 150 °C resulted in a reduction of 71% in fission track length or 65% reduction in apparent fission track density.

As α -recoil tracks fade more easily than fission tracks, the reduction in the density of the former during both geological phases must have been high. Such severe losses in the density of α -RT could easily reduce the recoil/fission track ratio sufficiently to create the impression that the expected 'excess' recoil tracks during the diffusion of lead or polonium isotopes had never occurred.

Our experimental results show that the diffusion of lead in mica along the cleavage planes is a rapid process. Hence, if our explanation of the lack of observable excess α -recoil tracks is accepted, the diffusion of lead isotopes from decay chains could well cause some of the polonium haloes. In that case ^{210}Pb is available (β^- , $T_{1/2}$ 22 yr), in one uranium series (^{238}U); and ^{211}Pb (β^- , $T_{1/2} = 38 \text{ min}$) in the other (^{235}U). Furthermore, the ^{232}Th series, which eventually produces ^{208}Pb , has ^{212}Pb with a half life of 19.6 h as a carrier.

We do not rule out the possibility that the diffusion of polonium itself may be a contributory—or even in some cases the main—cause. Experiments on this are in progress. Note that the three most commonly used minerals for halo investigations, biotite, fluorite and cordierite, all have cleavage properties; although, in the case of the cordierite, cleavage is not perfect. In

such materials, fast ion-conduction parallel to the cleavage planes could be expected and would probably be associated with high mobilities at elevated temperatures.

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Secondary batteries powered by forced ionisation

SECONDARY batteries normally store energy by accumulation of electrode products formed by electron transfer, that is, production of a reducing agent at one electrode and an oxidising agent at the other electrode. I have demonstrated a storage battery concept that depends on the energy associated with ionisation of slightly dissociated compounds, for example, forcing the equilibrium $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$ to the right stores energy. A cell for recovery of this energy as electricity is described here. De Körösy and Zeigerson have mentioned the possibility of a primary battery based on these principles¹.

The key element of such a cell must allow union of the ions at a fixed phase boundary. Removal of H^+ from one solution and OH^- from the other produces a potential which is related to the equilibrium constant for the reaction. The phases may be separated by a bipolar ion exchange membrane or a bipolar depolarising electrode. The cell configuration for each type of separator is shown in Figs 1 and 2. The flow of ions indicated is for the charging cycle and flow is reversed for discharge. The net process is: $\text{H}_2\text{O} + \text{MX} \rightleftharpoons \text{MOH} + \text{HX}$.

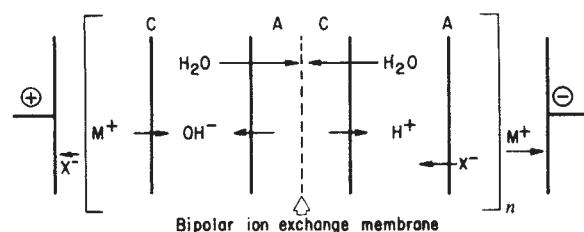


Fig. 1 Schematic for repeating cell in a forced ionisation secondary battery using a bipolar ion exchange membrane.

Bipolar ion exchange membranes have been made by lightly fusing a cation exchange membrane to an anion exchange membrane². The alternative procedure of de Körösy and Zeigerson¹ forms both the anion and cation exchange sites on opposite sides of a sheet of sulphochlorinated polyethylene. The use of bipolar ion exchange membranes for production of acid and base has recently been discussed in considerable detail^{3,4}. During this cycle, that is charging, inefficiency is associated with migration of either hydrogen or hydroxyl ions across the ion exchange barrier.

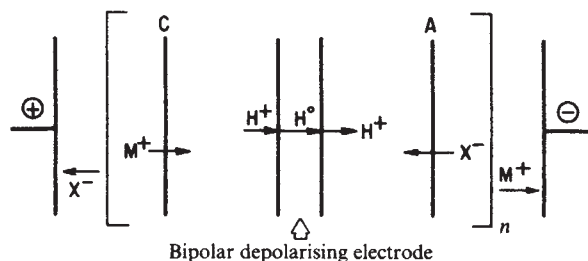


Fig. 2 Schematic for repeating cell in a forced ionisation secondary battery using a bipolar depolarising electrode. A, anion exchange membrane; C, cation exchange membrane.

A depolarising bipolar electrode has been used in this laboratory for production of an acid in a base. The same type of cell has now been used to produce electricity by neutralisation of acid and base. The cell is shown in Fig. 3. Hydrogen electrodes are used to convert the potential produced by migration of cations across the cation exchange membrane. The migration of hydroxyl ions to the acidic solution is a source of inefficiency as the potential is immediately nullified by the free migration of a sodium ion in the same direction.

The production of electrical energy from acid and base was carried out as follows. The entire system was placed under a positive pressure of hydrogen and the solution compartments filled with aqueous sodium phosphate. The central palladium foil was made cathodic and the end foils anodic⁵. This caused a net transport of hydrogen into the central palladium foil to speed equilibration with the external hydrogen. After equilibration acidic and basic solutions were passed in. The current-voltage relationships obtained are shown in Fig. 4.

These experiments demonstrate the use of two working fluids (water and methanol). As shown by methanol, compounds with lower ionic dissociation give higher cell voltage. The theoretical cell voltage associated with a number of equilibrium systems is calculated in Table 1.

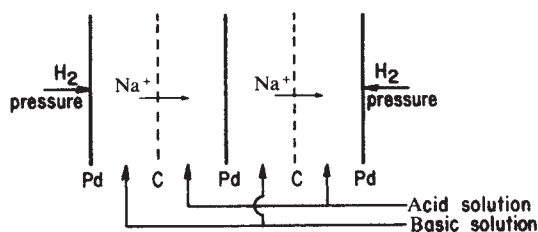
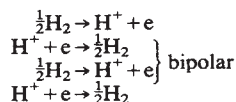


Fig. 3 Schematic for cell with net reaction $\text{H}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O}$. Electrode reactions, left to right:



Pd, activated palladium foil; C, Na ion cation exchange membrane.

The higher voltage systems place limits on the counter ions associated with the above ions of the charged state. These ions must move readily through ion exchange membranes and form ionic compounds with highly active ions. The alkali metal ions and the counter ions of superacids would be required in the most energetic systems. In addition, the salt formed by these ions must be sufficiently soluble to give good electrical conductivity. It is apparent that this scheme requires a bipolar member stable to extremely strong acids and bases. In these circumstances, the bipolar depolarising electrode may be more suitable than the bipolar ion exchange membrane. The latter are generally subject to degradation on the anion exchange side at extremely basic conditions.

The cells shown in Fig. 1 also require an anion exchange and a cation exchange membrane for each repeating unit. The anion exchanger operates between a salt solution and a strongly acidic solution. As a result, high efficiency for hydrogen ion rejection is necessary. However, chemical resistance will be less demanding than that in the corresponding part of the bipolar ion exchange membrane. Similar considerations apply to the cation exchange portions.

Table 1 Theoretical repeating cell e.m.f. for several working fluids as a function of the equilibrium shown

	-log Equilibrium constant	cell e.m.f.
$\text{HF} = \text{H}_2\text{F}^+ + \text{F}^-$	3	0.18
$\text{H}_2\text{O} = \text{H}_3\text{O}^+ + \text{OH}^-$	14	0.83
$\text{NH}_3 = \text{NH}_4^+ + \text{NH}_2^-$	35 (6)	2.06
$\text{CH}_4 = \text{CH}_5^+ + \text{CH}_3^-$	~60	~3.5

The high mobility of hydrogen and hydroxyl ions make high rejections of these ions particularly difficult. The use of any other working fluid with less mobile ions would be desirable from this point of view. However, such ions will add to the internal resistance of the bipolar ion exchange membrane.

The systems discussed all involve transport of H^+ or H^0 , but this is not essential. Any of a large number of liquids which yield ions under high electric field might be suitable. For example, the equilibria



are reported.

Such working fluids could greatly reduce the demands on the anion exchange component of the bipolar membrane. At the same time, the character of the cation exchange portion becomes more critical.

The metal foil depolarising bipolar electrode is obviously not suitable for such working fluids. The basic requirements for this component of the system are the same as those for gas depolarised electrodes—electronically conductive, liquid impermeable, but gas permeable. The surface should also be catalytic for the desired reaction, for example, $\text{Cl}^- \rightarrow \text{Cl}^0 \rightarrow \text{Cl}^+$. Such electrodes are readily built but any practical system requires a balance of mechanical strength and rapid gas permeability which is difficult to achieve.

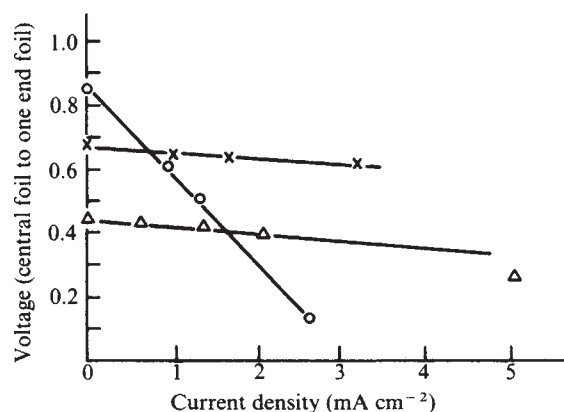


Fig. 4 Current-voltage plot for cells powered by the net reaction $\text{H}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O}$. x, 17% aqueous phosphoric acid, 20% aqueous sodium hydroxide; Δ , 20% aqueous sodium dihydrogen phosphate, 20% aqueous sodium hydroxide; $\square$, Phosphorus pentoxide in methanol 25 g l^{-1} , sodium methoxide in methanol 25 g l^{-1} . Current density was established by a variable external resistance.

At the ends of any assembly of cells of this type, electrodes which convert ionic charge to electronic charge are required. The hydrogen electrode was used in the above experiment. Electrodes such as those used in secondary batteries may be suitable. For example, nickel electrodes may be suitable for an aqueous cell provided they are placed in strongly alkaline compartments. The electrodes would operate on the reversible $\text{Ni}^{2+} \rightleftharpoons \text{Ni}^{3+}$ cycle with no net contribution to energy storage.

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Evidence for a higher natural uranium content in world rivers

α -SPECTROMETRIC uranium isotopes analyses on groundwater samples from the highly fertilised Handschuhsheimer Feld area in Heidelberg suggest that the uranium content of the groundwater is primarily of natural origin; uranium derived from phosphate fertilisers is adsorbed on the uppermost soil layers and the uranium content of the water is correlated to the HCO_3^- content. A similar relationship between uranium and HCO_3^- also applies for world rivers and lakes. We show here that this indicates that a low uranium content is due to low HCO_3^- contents and not to lower contamination levels. On the basis of this correlation the average natural uranium content of world rivers, for 35% of the water input to the ocean, was calculated to be $0.3 \mu\text{g l}^{-1}$.

The uranium content of river waters varies by about two orders of magnitude, from $0.04 \mu\text{g l}^{-1}$ for the Amazonas, to a few $\mu\text{g l}^{-1}$ (refs 1–5). This higher uranium content is, however, suspected to be partly of anthropogenic origin due to uranium derived from phosphate fertilisers⁷. In fact, compared with normal soil and rock material with a few p.p.m. uranium, phosphate fertilisers have up to 200 p.p.m. uranium. This fertil-

iser uranium is thought to contaminate the ground and eventually the river-water. Thus the question arises of whether the present-day estimates for the average uranium input by rivers to world oceans are representative of all geological time and therefore applicable to steady-state model calculations^{4,6}. However, several factors seem to contradict the assumption of a high uranium-pollution level of river water: a clear-cut relationship between the uranium and phosphate content of the water has not been found. Rivers show a significant ^{234}U excess, that is, the activity ratio $\text{AU} = ^{234}\text{U}/^{238}\text{U} > 1$ (ref. 7), whereas fertiliser uranium has an $\text{AU} \sim 1$, as in the case of soil and rock material. In addition, high uranium contents were also measured for some Indian rivers with catchment areas of low fertilisation⁸. Moreover, the uranium concentration in natural waters is assumed to be mainly controlled by the redox potential of the environment and by CO_2 (ref. 7).

To estimate the uranium contribution to groundwater by fertilisers and hence to evaluate the natural average uranium content of rivers, we have carried out uranium-isotope analyses on five groundwater samples from the highly fertilised area of the Handschuhsheimer Feld, Heidelberg, which has been used for horticulture for several decades (Table 1). Results are also presented here for three groundwater samples from an unfertilised forest area some 15 km out of Heidelberg, for the commercially available fertilisers, as commonly applied in this area (Table 2), and for a number of major German rivers, including the Neckar, that receives a groundwater contribution from the studied area (Table 1).

A 10-l sample was spiked with ^{232}U (in weak HCl solution), left at least 2 h for equilibration of uranium isotopes, then filtered (paper filter) and passed through a column containing 3 g of Hyphan-resin⁹ ($\text{pH} > 5$, flow-rate 40 ml min^{-1}). Back-extraction was achieved with 50 ml 2 M HCl. The pH of this solution was then adjusted to 4–5 with NH_4OH , uranium extracted into 20 ml TTA (0.1 M in benzene) and back-extracted into 20 ml 1 M HCl. The TTA extraction and back-extraction steps were then repeated. Adding concentrated HCl, the final aqueous solution was made 8 M and passed through a Dowex I resin to separate uranium from thorium isotopes. The following electroplating step and measurement of the uranium isotopes have been described elsewhere¹⁰. Counting times around 3,000 min made the counting errors smaller than 5%, even if the uranium content was as low as $0.1 \mu\text{g l}^{-1}$.

In this area of the Rheinebene, groundwater recharge of about 200 mm yr^{-1} has been estimated from the downward

Table 1 U, HCO_3^- , nitrate, phosphate, tritium content and activity ratio of the water samples from the Handschuhsheimer Feld area, the Südkreis Mannheim area, and for some major German rivers

Sample	U content ($\mu\text{g l}^{-1}$)	AU	HCO_3^- (mg l^{-1})	Depth (m)	Nitrate (mg l^{-1})	Phosphate (mg l^{-1})	Tritium (TU)
Entensee I	0.84 ± 0.06	1.62 ± 0.07	108.9	20	126	ND	187
Wiesenweg III	1.05 ± 0.04	1.97 ± 0.06	129.8	26	125	ND	113
Wiesenweg I	0.92 ± 0.04	1.52 ± 0.05	134.2	36	110	ND	—
Wiesenweg II	1.59 ± 0.05	1.57 ± 0.05	138.6	43	120	ND	—
Entensee II	—	—	—	60	—	ND	94
Entensee III	1.84 ± 0.07	1.59 ± 0.05	107.8	108	56	ND	54
Südkreis Mannheim							
Brunnen I	0.23 ± 0.01	1.28 ± 0.04	92	24	6	ND	—
Brunnen II	0.30 ± 0.01	1.00 ± 0.08	92	24	6	ND	—
Brunnen III	0.49 ± 0.01	1.10 ± 0.05	92	24	6	ND	—
Neckar (Heidelberg)	1.46 ± 0.04	1.49 ± 0.04	152.5	—	—	—	—
German rivers							
Rhein	2.48 ± 0.12	1.29 ± 0.07	222.65	—	—	—	—
Lahn	0.71 ± 0.04	1.91 ± 0.10	146.4	—	—	—	—
Mosel	1.80 ± 0.20	1.40 ± 0.10	213.5	—	—	—	—
Maas	1.20 ± 0.20	1.60 ± 0.20	176.9	—	—	—	—
Nahe	3.50 ± 0.20	1.60 ± 0.10	190.6	—	—	—	—

ND, not determined.

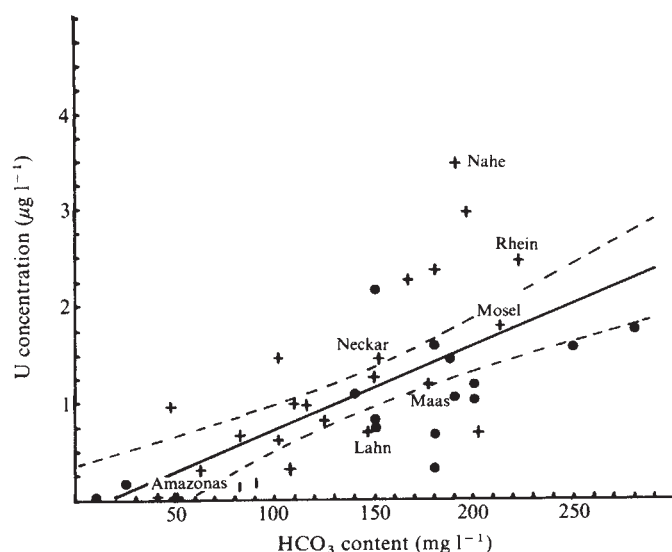


Fig. 1 Plot of the uranium content against HCO_3^- content for all world rivers and lakes, where both data are available. The circles indicate Texas rivers. The straight line is a linear fit for all values, the dotted lines show the 1σ deviation range. Apart from our values, U and HCO_3^- were not measured contemporarily (refs for the uranium contents are 1, 3–6 and 19–23, for the HCO_3^- values are 24, 26). The uncertainty in the HCO_3^- values exceeds 30% in some cases.

movement of precipitation water in the unsaturated zone, determined by tritium tracer experiments (sandy soil near Sandhausen-Heidelberg)¹¹. In addition a groundwater recharge of 190 mm yr^{-1} has been calculated from the bomb tritium vertical distribution in the shallow groundwater body of Sandhausen¹². In the Handschusheimer Feld, groundwater recharge by local rainfall is assumed to be about 113 mm yr^{-1} (data from Wasserwirtschaftsamt Heidelberg), as estimated using conventional hydrogeological methods. Both estimates agree with the bomb tritium standing crop in the shallow groundwater (see Table 1). At a fertilisation rate of $100 \text{ g m}^{-2} \text{ yr}^{-1}$, as commonly applied, some 12 g of nitrogen are annually incorporated to the soil. With an average groundwater

supply of 113 mm one can determine that some 25% of the fertiliser nitrate goes into the shallow groundwater, raising the natural nitrate concentration by some mg l^{-1} (as in the Südkreis Mannheim area) to values as high as 126 mg l^{-1} . (Water is used for irrigation only.) Although fertilisers have a high uranium content (Table 2) the uranium content of the shallow groundwater is even lower than that of the deeper groundwater, having lower nitrate and tritium contents. At the fertilisation rate of $100 \text{ g m}^{-2} \text{ yr}^{-1}$, taking into account an average uranium content of fertilisers of 50 p.p.m. , the contamination should be as high as 40 μg l^{-1} , if all uranium was transported in the newly formed groundwater.

The conclusion must be that more than 95% (2σ) of uranium supplied with the fertilisers are retained in the uppermost soil layers. This conclusion is corroborated by the fact that the activity ratio of the uranium isotopes $^{234}\text{U}/^{238}\text{U}$ of the groundwater lies around 1.6, and is similar to that of the Neckar, whereas uranium in fertilisers has an $\text{AU} = 1$; less than 0.01% of the phosphate goes into groundwater. Assuming a similar transport mechanism for uranium the contamination would make less than 0.004 μg l^{-1} . Although uranium is apparently released in only negligible amounts to groundwater, or river water, the uranium enrichment in the ploughed and therefore well mixed upper 20–30 cm soil layer should be no higher than 1 p.p.m. and therefore almost undetectable. The lithological profile of a length of 205 m consists of a sequence of water 2–15 m deep bearing gravel horizons interbedded with clays 5–10 m thick acting as aquicludes (data from Wasserwirtschaftsamt Heidelberg). Uranium analyses on samples from the borehole core Entensee showed normal uranium contents, ranging between 1 and 4 p.p.m.

In our groundwater samples the uranium content is fairly well correlated with the HCO_3^- content. A correlation between uranium and HCO_3^- has been reported for groundwaters from a small area of the UK¹³, for the uranium content in a salt lake in California¹⁴ and for mineral waters in the Taunus area¹⁵. This correlation should presumably be attributed to the water-soluble $\text{UO}_2(\text{CO}_3)_3^{4-}$ complex with a high formation constant in a range of $\text{EH} > -0.3$, $\text{pH} > 5$ and $\log P \text{CO}_2 > -4$ (ref. 16). Most rivers meet these conditions and in fact a broad correlation also results for world rivers and lakes, including Texas rivers where a high contribution of fertiliser uranium was suggested

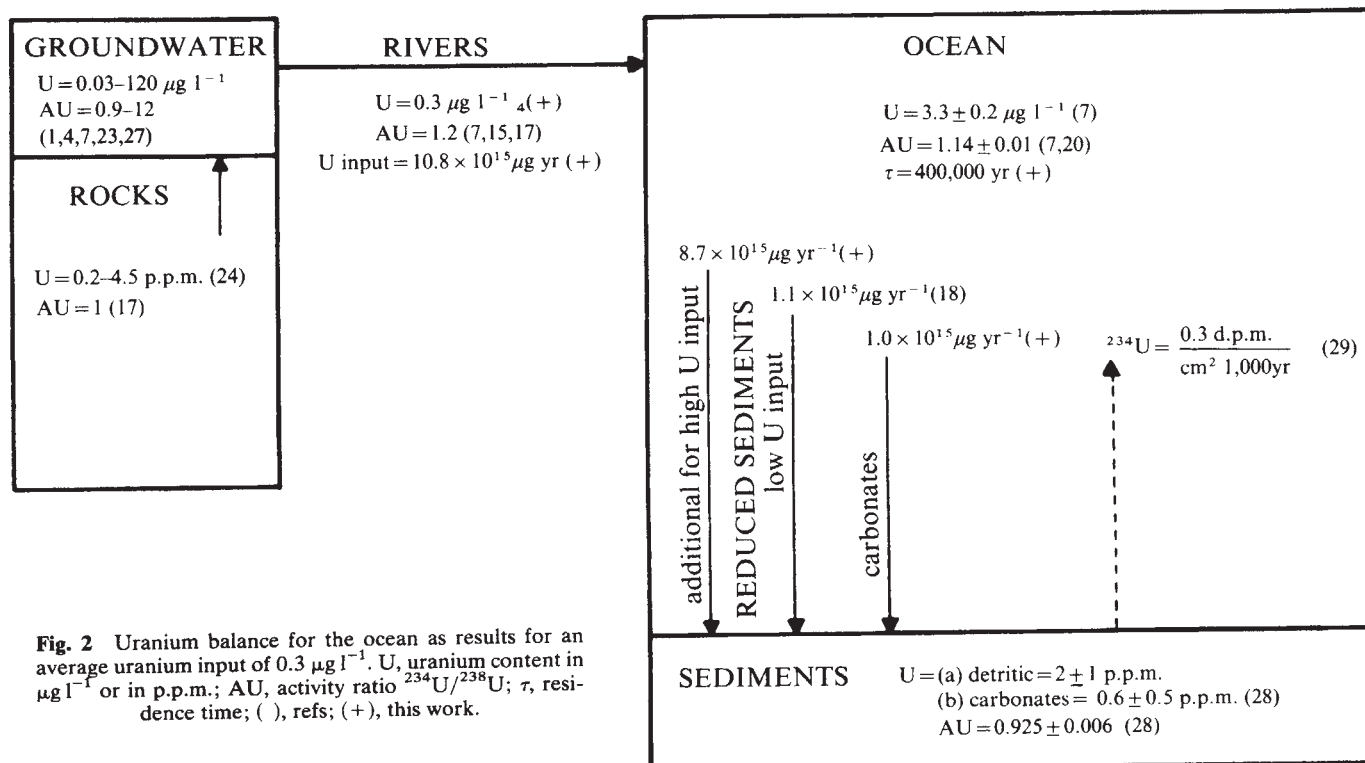


Fig. 2 Uranium balance for the ocean as results for an average uranium input of 0.3 μg l^{-1} . U, uranium content in μg l^{-1} or in p.p.m.; AU, activity ratio $^{234}\text{U}/^{238}\text{U}$; τ , residence time; (), refs; (+), this work.

Table 2 U content and AU of commercially available fertilisers

Fertiliser	Phosphate content	U content (p.p.m.)	AU
Kalksalpeter	Very low	0.3 ± 0.03	0.91 ± 0.1
Thomaskali	12%	3.3 ± 0.3	0.95 ± 0.1
Orgamin	6%	10.8 ± 2.0	1.05 ± 0.15
Nitrophoska-Blau	12%	48.8 ± 2.0	1.00 ± 0.04
Nitrophoska	12%	50.3 ± 2.0	1.01 ± 0.04

Only the nitrophoska fertilisers are usually applied in the Handschusheimer Feld area.

(Fig. 1). Thus the uranium content largely depends on the available HCO_3^- in the water, giving evidence that the recent uranium content in the rivers is primarily of natural origin. Differences in the bedrock material from which uranium is being leached and uranium derived from fertilisers are obviously of less importance. They might be responsible for the large scattering in Fig. 1, together with the large uncertainty of the HCO_3^- values, not contemporaneously measured with uranium. In the light of these results it seems that the low uranium content of the Amazonas is due to its unrepresentatively low HCO_3^- content and not to a lower level of anthropogenic contamination.

On the basis of this correlation we estimate the natural uranium input to the ocean, for some 35% of the supplied water, to be $0.3 \mu\text{g l}^{-1}$, in agreement with Turekian's previous estimate³. The average activity ratio of the supplied water is ~1.2 (refs 7, 15, 17). Applying these values to a steady-state model we calculate a residence time for uranium of 400,000 yr and an annual removal to the sediments of $10.8 \times 10^{15} \mu\text{g}$, a value some 10 times higher than when the Amazonas content is taken as the average. A roughly calculable part of uranium is deposited with the marine carbonates. The rest should then be incorporated into anaerobic sediments, which are deposited mainly on the shelf. We calculate a uranium deposition of $500 \mu\text{g cm}^{-2}$ per 1,000 yr for the entire, and $30 \mu\text{g cm}^{-2}$ per 1,000 yr, for some 6% of the shelf area. The latter is similar to the uranium-deposition rate given by Veeh for the Walfish Bay area, South-west Africa¹⁸.

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Distribution of rare earth elements in some North Atlantic Kimmeridgian black shales

RARE earth element (REE) patterns of Upper Jurassic, Kimmeridgian, shales from various localities are shown here to have only minor variation in spite of deriving from very different source rocks. The formation of such uniform patterns indicate that these sediments are mature, well mixed and formed in similar depositional environments. An extension of the North Sea Kimmeridgian petroleum source rocks is indicated. Minor differences in REE patterns can be related to variations in the sand/silt content.

The REEs from La to Lu ($Z = 57–71$), have been the basis of many geochemical studies. Most of these have considered hardrock problems, but several studies have also been carried out on sediments and sedimentary rocks. Sedimentary deposits often seem to have REE distributions reflecting the different source rocks, sedimentary processes and grain size variations^{1,2}, and some of the REE distributions have been related to REE fixation in marine depositional environments^{3,4}. Generally the

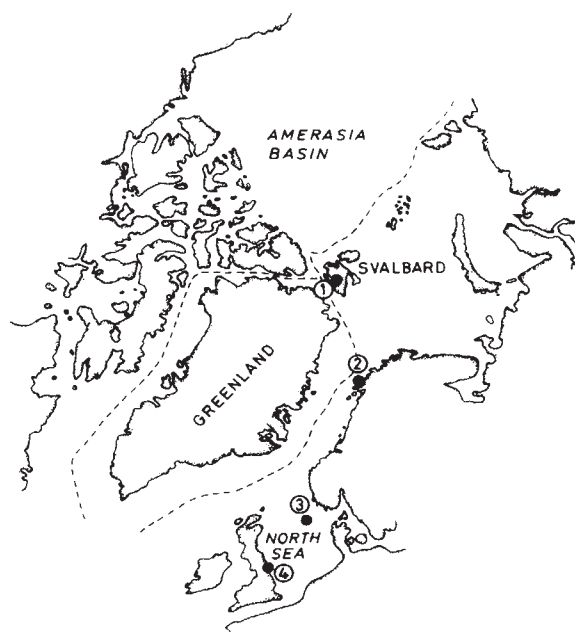


Fig. 1 Reconstruction of the Arctic and adjacent areas before the lower Tertiary opening of the Norwegian Sea. Stippled lines indicate Tertiary plate boundaries. (The compilation is taken from refs 14–17). ① Wimannfjellet, Svalbard; ② Andøya, Northern Norway; ③ Well 17/11-1, North Sea; ④ Yorkshire, UK.

REEs have rather short residence time in the oceans and relative enrichment in the heavier REE (HREE) is common^{3,5}. Because HREE form complexes with higher stability than the lighter REE (LREE), LREE have a shorter residence time in seawater and may be preferentially adsorbed on clay minerals⁴. Alteration and fractionation of the REE patterns may occur in weathering horizons⁶ in addition to variation due to fixation of REE from seawater. Weathered rocks are generally enriched in total REE compared with the original unweathered rocks⁷. Of the main constituents of sediments and sedimentary rocks, the clay fraction (clay minerals and colloids mainly) contains more REE than the sand/silt fraction (rich in quartz and feldspars)⁸. Separations of different sedimentary fractions, either by varying depositional conditions or by weathering reactions may lead to fractionation of the REEs.

REE analyses are normally presented in 'comparison diagrams' (ref. 9), where the elements analysed are all related to a reference distribution. Sedimentary and metamorphic rocks are generally compared with proposed upper crustal average, based on composite sample of 40 North American Shales (40 NAS)^{2,10}. The samples analysed here have been correlated

with this composite sample. The individual element/element ratios have been plotted on a logarithmic scale against atomic number (Fig. 2).

The present work was designed to study how different source rocks affect the REE compositions of the sediments of shallow marine depositional regimes. Radiochemical neutron activation analyses (RNAA) were carried out on 22 samples from the following localities (Fig. 1): Svalbard, Wimannfjellet; Northern Norway, Andøya; North Sea, Well 17/11-1, Norwegian sector; UK, Yorkshire. The method used is based on USGS standards G2 and BCR 1, using the recommended values of Flanagan¹².

In Upper Jurassic Kimmeridgian times similar depositional regimes existed in these four areas (Fig. 1). The four sections represent original different positions in the rather shallow epicontinental Kimmeridgian sea. However, different source rocks were exposed in the adjacent areas. The sediments deposited were generally dark, marine, organic-rich clays, although those on Andøya were somewhat more sandy/silty than in the other localities.

Eight (La, Ce, Nd, Sm, Eu, Tb, Yb, Lu) of the REEs have been determined (Table 1), distribution patterns are shown in

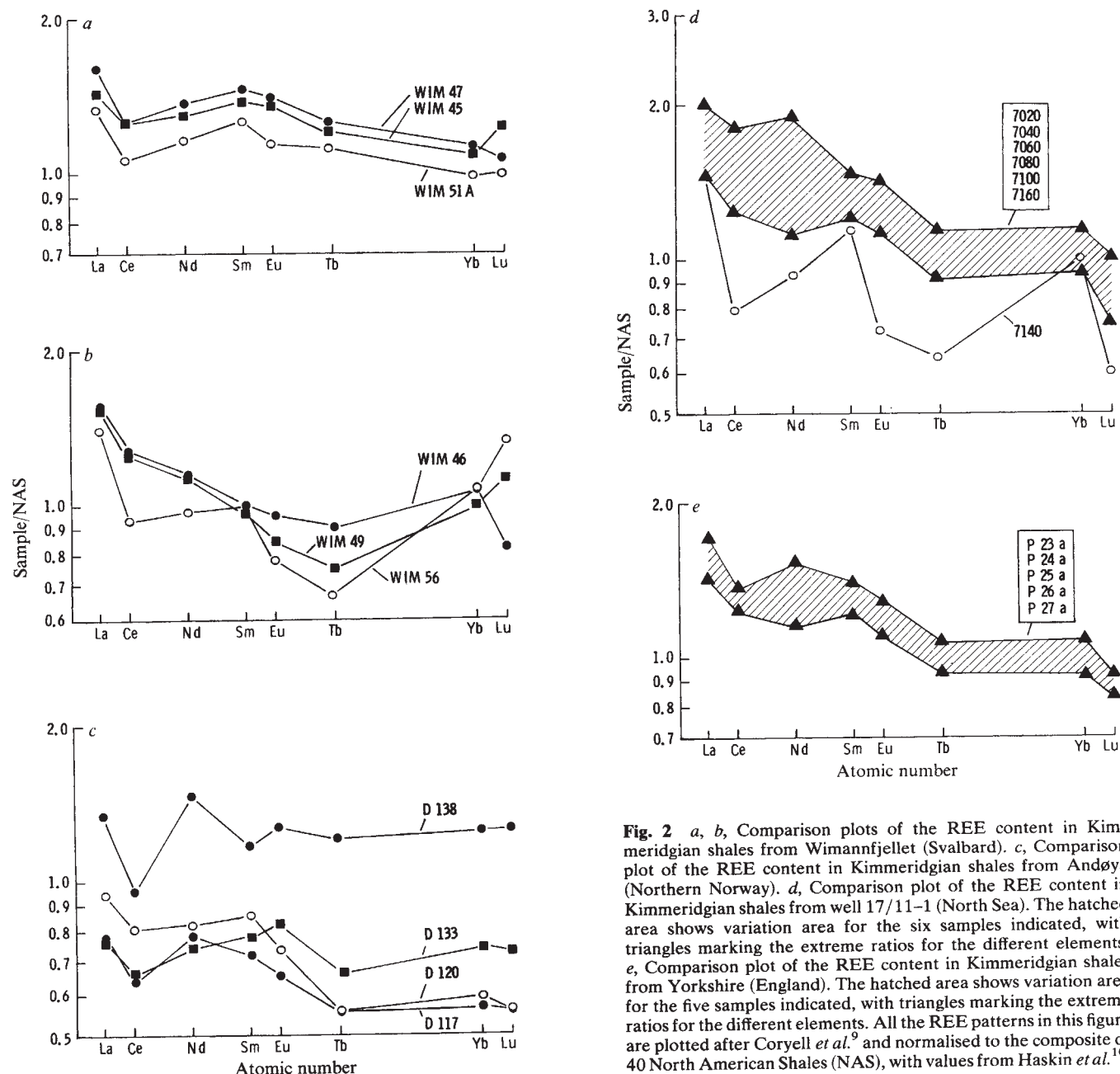


Fig. 2 *a, b*, Comparison plots of the REE content in Kimmeridgian shales from Wimannfjellet (Svalbard). *c*, Comparison plot of the REE content in Kimmeridgian shales from Andøya (Northern Norway). *d*, Comparison plot of the REE content in Kimmeridgian shales from well 17/11-1 (North Sea). The hatched area shows variation area for the six samples indicated, with triangles marking the extreme ratios for the different elements. *e*, Comparison plot of the REE content in Kimmeridgian shales from Yorkshire (England). The hatched area shows variation area for the five samples indicated, with triangles marking the extreme ratios for the different elements. All the REE patterns in this figure are plotted after Coryell *et al.*⁹ and normalised to the composite of 40 North American Shales (NAS), with values from Haskin *et al.*¹⁰.

Table 1 REE (p.p.m.) of Kimmeridgian shales from the North Atlantic region.

	Sample	La	Ce	Nd	Sm	Eu	Tb	Yb	Lu
Svalbard	WiM 45	45.8	93	42.8	7.81	1.69	1.03	3.41	0.60
	WiM 46	51.0	93	38.3	5.70	1.18	0.77	2.88	0.58
	WiM 47	51.1	94	45.8	8.37	1.75	1.08	3.47	0.57
	WiM 49	49.2	88	37.0	5.55	1.05	0.63	3.10	0.55
	WiM 51A	43.2	78	38.2	7.27	1.41	0.94	3.08	0.48
	WiM 56	44.9	67	32.0	5.71	0.98	0.57	3.38	0.64
Andøya	D 133	24.6	48	24.6	4.43	1.03	0.56	2.29	0.35
	D 117	25.1	47	25.6	4.16	0.81	0.47	1.74	0.26
	D 120	30.1	59	26.9	4.83	0.91	0.48	1.83	0.27
	D 138	43.2	70	48.4	6.74	1.59	1.02	3.82	0.60
North Sea	7020	46.9	96	37.2	7.40	1.41	0.81	3.24	0.46
	7040	50.7	102	53.2	6.92	1.51	0.91	2.91	0.43
	7060	64.1	121	62.7	8.54	1.75	0.96	3.12	0.45
	7080	50.1	91	44.2	6.98	1.42	0.78	2.65	0.36
	7100	52.8	98	42.1	8.28	1.51	0.88	3.53	0.43
	7140	46.8	59	30.7	6.63	0.89	0.56	3.00	0.29
	7160	52.5	100	47.4	7.68	1.47	0.90	3.47	0.49
Yorkshire	P 23a	54.3	95	50.2	7.98	1.61	0.91	3.24	0.44
	P 24a	48.3	94	42.2	7.47	1.45	0.82	2.95	0.43
	P 25a	49.4	91	40.6	7.72	1.57	0.84	2.88	0.42
	P 26a	47.8	100	49.2	7.02	1.37	0.79	2.84	0.44
	P27a	45.1	94	38.3	6.90	1.45	0.85	3.33	0.40
	NAS	32	73	33	5.7	1.24	0.85	3.1	0.48

NAS is a composite of 40 North American Shales values from Haskin *et al.*¹⁰.

Fig. 2. The patterns of the different samples are very similar. In spite of deposition at different localities and derivation from different sources, no major variations in the REE distributions are found (Fig. 2). This probably reflects rather extensive weathering, repeated reworking and homogenisation of sediments before final deposition as fine-grained silts and clays. The sample sets from the four different localities generally show minor enrichments in LREE. This may be due to minor selective adsorption of LREE on weathered clay minerals in the seawater.

In detail the samples from Yorkshire (Fig. 2e) and the North Sea (Fig. 2d) have higher total REE contents and show enrichment of LREE compared with samples from Andøya (Fig. 2c) and Svalbard (Fig. 2a, b). This correlates with certain mineralogical differences—the sediments in Yorkshire and the North Sea being generally fine grained, containing less quartz and feldspar. Higher sand/silt concentrations in the Andøya samples have served to dilute the samples which have lower REE concentrations.

The Svalbard samples analysed are, based on their REE distribution patterns, divided into two different populations (Fig. 2a, b). A selective leaching of the middle REEs due to diagenetic processes is the only explanation we can propose for this observation.

In Svalbard, Andøya and North Sea areas the Upper Jurassic transgression¹³ is reflected in a progressive increase in finer material towards the top of the Kimmeridgian. These developments are partly seen in an upwards increase in total REE and in lower LREE/HREE ratios, reflecting influx and deposition of finer-grained material with lower percentage of sand and silt. However, the similar REE patterns in the different localities clearly indicate rather persistent depositional conditions. Only sample 7140 in the North Sea sample set and sample D 138 from the Andøya sample set seem not to conform. These variations may be explained by occurrences of dolomite in D 138 which is not detected in the other Andøya samples, while sample 7140 from the North Sea have higher sand/silt and lower carbonate contents than other samples from that locality.

Strong weathering of source rocks and later reworking of weathering products resulted in deposition of marine clays with rather similar REE distribution patterns in the different localities, in spite of derivation from different source rocks. This

shows rather similar and persistent depositional regimes in the huge Kimmeridgian epicontinental sea and may indicate possibilities for Kimmeridgian petroleum source rocks north of the North Sea. The generally weak LREE enrichments point towards selective LREE adsorption on clay minerals in seawater. Minor variations in the REE concentrations may be due to grain size variation which in turn reflect minor differences in sedimentation conditions in the Kimmeridgian epicontinental sea.

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Losses of nitrous oxide dissolved in drainage water from agricultural land

DISCUSSIONS on the fate of nitrogen in soil usually assume that the gases produced by denitrification—nitrous oxide and molecular nitrogen—are lost only by movement in the gaseous phase towards the soil surface, where they escape to the atmosphere. As far as we know, there are no published reports of the transport of these gases dissolved in water draining from agricultural land. However, the anaerobic conditions necessary for denitrification develop to an appreciable extent in drained agricultural soils when the soil water potential is zero or positive¹⁻⁶; there is then likely to be drainage of water through the soil. In the soils of southern England, nitrous oxide can be found for extended periods during the wetter months^{7,8}, where there is on average ~250 mm rainfall in excess of evapo-transpiration and soil storage⁹. As nitrous oxide is fairly soluble in water (1.0 ml gas per ml water at 5°C) we have examined the possibility that significant quantities are transported from the soil in drainage water. We report here for the first time observations that nitrous oxide can be lost from the soil by leaching in amounts comparable with those lost in the gas phase from the soil surface.

Water issuing from agricultural drains in heavy clay soils (Table 1, site 2 Lawford (Rowsham) series¹⁰, sites 3 and 4 Denchworth series¹¹) contained much more nitrous oxide than was present in rainfall. Appreciable concentrations of nitrous oxide were also found in water draining from lysimeters containing intact columns (80 cm diameter and 135 cm deep) of two other fine textured soils (Salop¹¹ and Bromyard¹² series). Laboratory incubation experiments showed that the denitrifying activity of drainage water was negligible, even when nitrate and

glucose were added in anaerobic conditions (unpublished results). The nitrous oxide in drainage water had, therefore, come from the soil and not from biological activity in water as it passed down the drain. In addition to water from agricultural drains, appreciable concentrations of nitrous oxide were found in samples from streams, a lake and domestic water supplies (Table 1), but some of the nitrous oxide contained in these sources may possibly have been produced after the water had emerged from the drains (for example, as a result of microbial activity in bottom sediments¹³).

A preliminary estimate of the quantity of nitrous oxide that might be contained in drainage from our experimental fields can be made by assuming that all the excess of rainfall over evapo-transpiration during the winter months (November to March inclusive), on average about 250 mm of water, was removed from the soil by the tile drainage system, and that also this drainage contained a concentration of 100 $\mu\text{g N}_2\text{O-N l}^{-1}$; the amount of nitrogen leached as nitrous oxide would then be about 0.25 kg ha^{-1} , but greater rates of loss may be expected during short periods of high rates of denitrification and drain flow. In comparison the fluxes of nitrous oxide gas from the soil surface in winter, measured at the same sites and during the same period as those in drainage were equivalent to 0.15–0.9 kg N ha^{-1} (ref. 14). Losses as molecular nitrogen in the gas phase from the soil surface may be considerably greater than this, as it is commonly believed¹⁵ that the ratio of $\text{N}_2:\text{N}_2\text{O}$ is ~16:1. However, leaching losses of molecular nitrogen greater than that of nitrous oxide are unlikely because the solubility of nitrogen in water is 50 times less than nitrous oxide (0.02 ml gas per ml water at 5°C) and it would therefore require an unusually high $\text{N}_2:\text{N}_2\text{O}$ ratio of 50:1 or more to achieve comparable rates of loss. For comparison, the concentration of nitrate-N in drainage water always exceeded the concentration of dissolved nitrous oxide-N by 1–3 orders of magnitude (Fig. 1).

Water samples collected from an open drain outlet, as in the present work, provide an inaccurate basis for estimating the amounts of nitrogenous gases leached from the soil, as an

Table 1 Nitrous oxide content of water from various sources

Source of water	Site*	Sampling period	No. of sampling occasions	Concentrations observed	
				Mean ($\mu\text{g N}_2\text{O-N l}^{-1}$)	Range
Agricultural drains	1	Dec 1977–May 1978	41	8.1	2–25
	2	Feb 1977–Apr 1977	7	21.4	4–56
	3	Nov 1977–May 1978	60	21.4	4–132
		Dec 1977–Jan 1978	2	8.7	1–27
Lysimeter drainage	4	Dec 1977–May 1978	20	108.2	0.7–415
Soil solution	1	Nov 1977–May 1978	17	15.1	0.3–544
	2	Oct 1975–Apr 1976	25	1.2	0.3–28
		Dec 1976–Jul 1977	18	277.3	0.5–9984
		Nov 1977–Mar 1978	17	92.8	0.3–1405
Bore-hole in chalk	5	June 1978	2	32.2	4–56
Domestic supplies	4	June 1978	2	53.5	24–83
	5	June 1978	2	20.6	7–61
Surface waters	4 (Lake)	June 1978	1	9.4	–
	4 (Stream)	June 1978	1	7.1	–
	1	Jan 1978	1	4.3	–
	2	Jan 1978	1	3.6	–
Rainfall	4	June 1978	1	0.3	–

Water was sampled using 5-ml glass syringes sealed with 3-way plastic valves. Samples were analysed using an equilibration technique¹⁶ on a Pye-Unicam GCV gas chromatograph equipped with an electron capture detector (350°C) and a column (2 mm wide, 100 cm long) of Carbosieve B at 110°C.

* Sites: 1, Northfield, near Stanford-in-the-Vale, Oxfordshire; 2, Compton Beauchamp, Uffington, Oxfordshire; 3, MAFF Experimental Husbandry Farm, Drayton, near Stratford-upon-Avon, Warwickshire; 4, ARC Letcombe Laboratory, Wantage, Oxfordshire; 5, Mill Farm, Marten, Marlborough, Wiltshire.

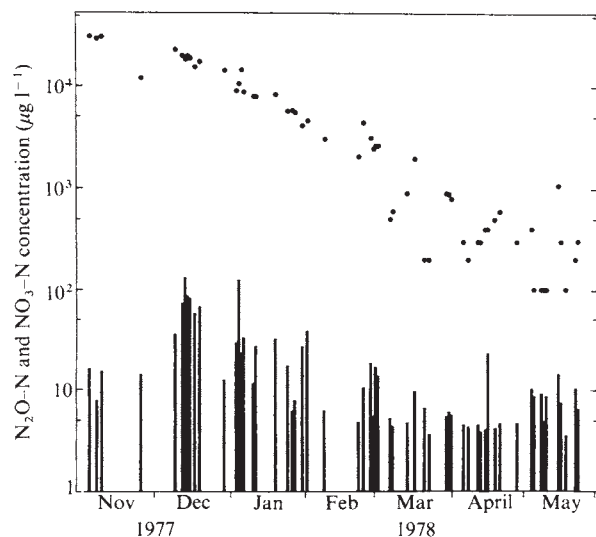


Fig. 1 Nitrous oxide (solid bars) and nitrate (points) content of water draining from an arable field during the winter of 1977–78 at Compton Beauchamp, Uffington, Oxfordshire. The field was sown with winter wheat in October 1977; no nitrogen was used until April 1978 when 80 kg N ha⁻¹ as ammonium nitrate was applied. The rainfall between November 1977 and May 1978 was 440 mm (the long term average for this period is 405 mm).

unknown amount of gas diffuses from the water or soil into the atmosphere of the drain. Air drawn from drains contained nitrous oxide concentrations of up to 100-fold that in the terrestrial atmosphere, but neither measurements nor estimates of loss by gas movement in drains have been made. However, improved estimates of leaching losses of nitrous oxide made during a number of contrasting winters may be obtained from a long term field experiment, begun in 1978, where the equipment for the continuous monitoring of water is designed to prevent the gas phase transport of nitrous oxide from the drain.

Although in our preliminary experiments the loss of nitrogen from the soil profile by nitrous oxide dissolved in drainage water seemed a minor component of the soil nitrogen balance, in other conditions losses by this route could be larger, particularly where high rates of denitrification and drainage of water are experienced. However, the loss of nitrous oxide in drainage from agricultural land is an important new factor to be considered in assessments of the contribution made by denitrification to the nitrous oxide content of the atmosphere.

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Developmental fate of the cyanogenic glucoside linamarin in Costa Rican wild lima bean seeds

WHEN a seed germinates, we expect the defensive secondary compounds it contains to be transferred intact to the growing seedling, or variously decomposed to produce resources for the growing seedling. The seeds of wild indigenous Costa Rican lima beans (*Phaseolus lunatus* L.) contain about 3.45% fresh weight of linamarin¹, a cyanogenic glucoside that can enzymatically decompose to produce 0.37% fresh weight hydrocyanic acid (HCN). We report here that, after germination, the amount of linamarin in the total seedling (roots, cotyledons, and shoots) remains essentially constant during a 26-day experimental period and moreover corresponds to the amount of linamarin present in the seed before germination (Fig. 1).

To determine the fate of this defensive secondary compound in young seedlings, we germinated seeds and monitored the linamarin content of the seedlings for 26 days. While the amount of linamarin per seedling remained constant, the distribution of the glucoside present initially only in the seed changed dramatically with time. For example, after 6 days 42% of the total linamarin in the seedling was contained in the cotyledons, 27% was in the new leaves and shoot tip, 20% was in the stem and 11% was in the roots. After 14 days, 69% of the linamarin was in the leaves, and after 16 days the cotyledons were devoid of linamarin and dropped off. The change in fresh weight of the different plant parts, expressed as per cent of the plant's total weight, paralleled the change in linamarin for each different part. That is, the cotyledons decreased in weight each day by an

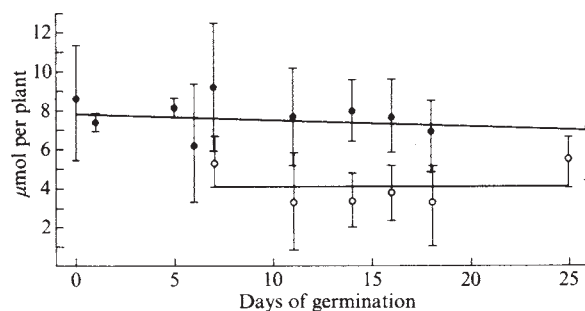


Fig. 1 The linamarin content of individual seeds or seedlings of wild lima beans (*Phaseolus lunatus*). Seeds were germinated by pricking the thin seed coat with a pin and soaking overnight in distilled water. Seedlings were grown in vermiculite at 24 °C with a 14-h photoperiod. On the 7th day of growth the cotyledons were carefully removed from 15 seedlings (○) to permit comparison with untreated seedlings (●) whose cotyledons remained on the plant for ~2 weeks. Linamarin content was determined by grinding weighed fresh seeds or seedlings in liquid nitrogen with a mortar and pestle. The ground sample was immediately transferred to 2 ml of 0.1 M phosphate buffer (pH 6.8) containing the enzymes emulsin and linamarase in the outside compartment of a centre well flask; the centre well contained 1 ml of 1 M NaOH. After 24 h in a shaking water bath at 37 °C, the contents of the centre well were assayed for HCN, a product of the enzymatic decomposition of linamarin. The method used⁶ detects HCN in the range 0.01–0.15 µmol. Qualitative examination of 80% (v/v) ethanolic extracts by gas liquid chromatography⁷ established that linamarin was the only cyanogenic glycoside present either in seed or seedlings. Seeds were hand-shelled from mature dry pods collected from a large wild plant in March, 1976 at Finca La Pacifica, near Cañas, Guanacaste Province, Costa Rica. An average seed weighed 62 mg (s.d. 13 mg, n = 25). Seeds from this plant were polymorphic; 60% were black and 30% brown. The black seeds tended to be slightly heavier, to have a higher instantaneous germination rate, and to possess a thicker seed coat than the brown seeds. Only black seeds were used in the study reported here.

amount corresponding to 5% of the total weight of the seedling and lost 4.4% of the total linamarin each day. Roots gained 2% in weight and 0.5% in linamarin each day while stems lost 0.9% in weight and 0.4% in linamarin each day.

The data displayed in Fig. 1 suggest that the plant either transfers its linamarin intact from the seed (cotyledons) or breaks it down and resynthesises it in equal amounts during new growth of the seedling. To determine what happens, the cotyledons were removed from 15 plants after 7 days of growth. The excised cotyledons contained approximately 4.5 μmol linamarin, and the glucoside content of the seedlings dropped to about 4.0 μmol per plant. Although the treated seedlings continued to grow and develop, their linamarin content remained constant throughout the experiment. This could be explained by assuming that the plant uses only the breakdown products of the linamarin for resynthesis in the growing seedling. However, the breakdown products of the aglycone of linamarin are acetone and HCN, compounds which are not readily converted to valine, the demonstrated primary precursor of linamarin in other linamarin-containing species¹. We therefore prefer the more parsimonious explanation that the linamarin is transferred intact from the cotyledons to the growing seedling.

The degree to which plants synthesise secondary compounds as they change and develop, as opposed to shifting the location of compounds already synthesised, should be an important consideration in the pattern and budget of chemical defenses against herbivores. In *Heteromeles arbutifolia*, a chaparral evergreen shrub in California, the cyanogenic glucoside in the immature fruit is transferred to the ripening seed, and thus the same chemicals probably serve as a defence in consecutive developmental stages². On the other hand, seeds of *Sorghum vulgare* do not contain a cyanogenic glucoside but the seedlings synthesise large amounts of it (dhurrin) in the first 5 days of growth³. Some kinds of secondary compounds are probably removed from deciduous tree foliage before the leaves are shed; presumably they can be used in other parts of the plant or in leaf production in later seasons.

Seeds contain a large variety of secondary compounds which are undoubtedly defensive for the seed⁴, amongst other possible functions. While it is easy to postulate that such secondary compounds are stored as resources for developing seedlings, this has never been demonstrated. In the present study we have demonstrated that they may also be defense resources for the developing seedling. In short, if the seed is a container whose volume or weight is to be minimised (for dispersal purposes), the parent plant produces a higher quality package for the seedling if it puts the intact, defensive molecule into the seed than if it puts in the raw materials for synthesis of that molecule.

With this view in mind, there are at least three selective forces acting on the quantity of a given kind of secondary compound to be found in a seed. First, there are the levels that the parent plant can donate considering the total number of seeds it is producing and its own needs. Second, there are the levels necessary to deter the relevant herbivores from eating the seeds. Third, there are the levels needed to give enough defensive compounds to the seedling to meet its herbivore challenges. The first selective force will act to minimise the compound level and the second two to maximise it; however, the second two need not necessarily have the same optimal level. For example, co-evolutionary selection by a bruchid beetle known to feed on lima bean seeds (D.H.J., unpublished) may drive the concentration of linamarin in the seed up until it is higher than that which is needed by the seedling. At this point, the levels of linamarin in the seedling may have increased to a point well over that which is needed to deter an insect that eats seedling leaves. Conversely, strong selection on the seedling for increased linamarin content by a defoliator may drive the linamarin content of the seed to a high enough level that the bruchid larvae can no longer survive in the seed. This would be a real-life example of the theoretical manner in which herbivores competitively interact through the medium of the resource budget of the plant in evolutionary time⁵.

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Somatic fusion between cell wall mutants of *Chlamydomonas reinhardtii*

SOMATIC FUSION has recently been used to isolate hybrids from auxotrophic mutants of fungi^{1,2}, yeast³ and mosses^{4,5}. These fusions were performed by using polyethylene glycol (PEG) and/or CaCl_2 solutions on protoplasts which were prepared from cells of tissues treated with enzyme mixtures to dissolve the cell wall. In the unicellular green alga *Chlamydomonas* mutant strains unable to make a cell wall have been isolated^{6,7}. Such mutants, similar to plant protoplasts prepared by enzymatic digestion, can be easily grown in defined liquid or agar media⁸. However, they differ from true protoplasts in that they do not burst in hypotonic medium or even in distilled water, indicating that some mechanism of osmoregulation is present⁶. It was thus of interest to determine whether these cell wall mutants could be used as plant protoplasts in fusion experiments. We report here the isolation of stable fusion products

Table 1 Comparison of four fusion products from 303 $\times$ 308 with the wild-type haploid strain and a diploid *arg-7* $\times$ *arg-3* obtained by sexual mating

Strain	Mating type	Cell volume* (μm^3)	Nuclear volume† (μm^3)	Relative DNA content§		Chloroplast DNA (% of total DNA)
				1	2	
Haploid (WT)	+ or -	30 (± 5)	2.8 (± 0.5)	1.0	1.0	14.1
Diploid (<i>arg-7</i> $\times$ <i>arg-3</i>)	-	58 (± 10)	6.9 (± 1.9)	1.9	1.9	11.1
303 $\times$ 308/1	+	58 (± 10)	7.1 (± 1.2)	1.8	2.0	7.3
303 $\times$ 308/2	+	56 (± 10)	-	-	-	-
303 $\times$ 308/3	+	56 (± 10)	7.6 (± 1.4)	-	-	-
303 $\times$ 308/4	+	60 (± 10)	-	-	-	-

* Cells grown on agar medium were suspended in distilled water and incubated overnight in the dark before being measured. The volumes were determined with a Coulter electronic counter model ZF (orifice 100 μm).

† Cells grown on agar medium were fixed and stained for nuclei (Feulgen reaction). The nuclear volumes were determined from diameter measurements of 20 nuclei after photographic magnification, assuming that nuclei were spherical.

§ Cells were grown in 300 ml of liquid medium and collected during exponential growth. 1, The DNA was estimated by the diphenylamine reaction (modification¹² of the Burton's method). Values are means of two experiments. 2, Cells were incubated overnight in distilled water then fixed in alcohol. They were treated with RNase for 1 h (1 mg ml⁻¹ at 37 °C) and labelled with propidium iodide. The DNA content was measured by flow microfluorometry.

|| The cells were grown in 1 l of liquid medium. They were lysed with SDS (10% final concentration) and then DNA were extracted by successive digestions with pronase, RNase and proteinase K, interspersed with alcohol precipitations¹³. The DNA were further purified on agarose¹⁴ and ultracentrifuged in CsCl gradients. Nuclear ($d = 1.724 \text{ g cm}^{-3}$) and chloroplast ($d = 1.695 \text{ g cm}^{-3}$) were clearly separated and analysed by UV spectrophotometry.

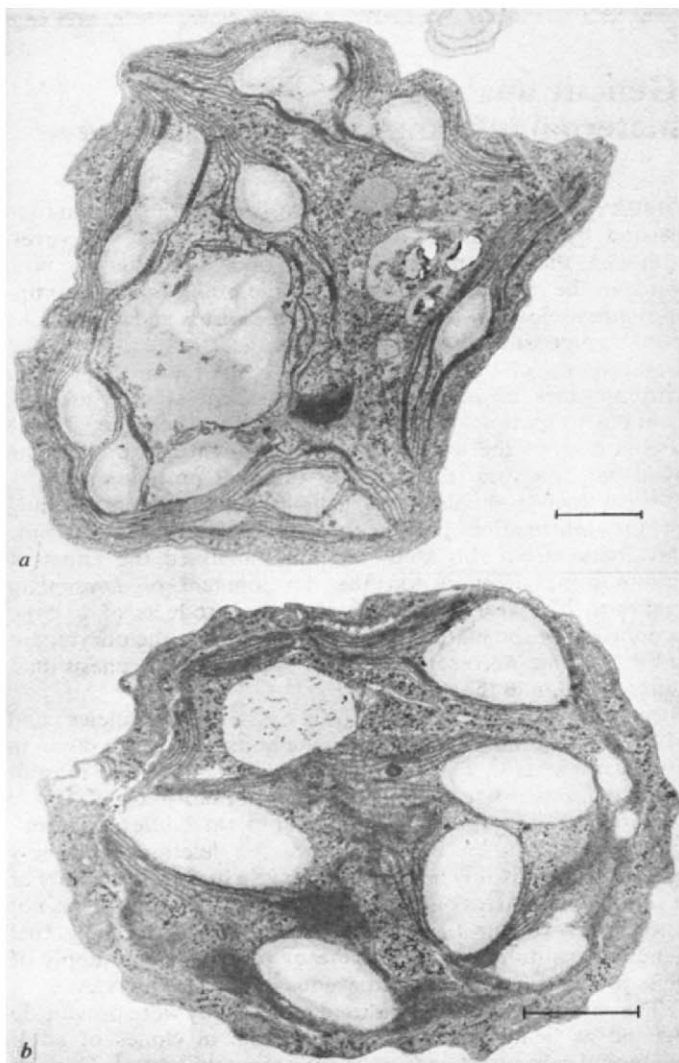


Fig. 1 Ultra-thin sections through cells of 303 (a) and 308 (b) strains lacking a cell wall. The cells were fixed with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.0, for 1 h, post-fixed in 2% osmic acid for 2 h. The samples were dehydrated through an alcohol series and embedded in Epon. Sections were stained with uranyl and lead. Scale bar, 1 μ m.

obtained from cell wall haploid mutants of *Chlamydomonas reinhardtii* having the same mating type (mt^+) and bearing genetic markers of auxotrophy.

The two following strains, isolated after crosses between mutants available in our laboratory, were used in the fusion experiments: strain 303, mating-type plus (mt^+), absence of cell wall ($CW15$)⁶, auxotrophy for arginine ($arg-7$); strain 308, mating-type plus (mt^+), Cw_d (a putative cell wall strain defective for four phosphatases and called $P_2P_dPD_dPD_d$ by Loppes and Deltour⁷), auxotrophy for arginine ($arg-7-3$). The $arg-7$ and $arg-7-3$ mutants, both defective for the same enzyme and mapping in the same cistron are able to complement and restore the wild phenotype in diploids⁹.

Analysis of the strains 303 and 308 under the light microscope revealed that both were deprived of a cell wall. The absence of wall was confirmed in electron microscopy (Fig. 1).

A complementation test made between the strain 308 mt^+ and a strain identical to 303 but mt^- led to recover prototroph diploid cells with a normal cell wall. This indicates that $CW15$ and Cw_d mutations do complement and are probably located in different genes.

The mt^+ cells used in the fusion experiments were routinely grown on agar mineral medium deprived of ammonia and enriched with yeast extract (M-N + YE¹⁰), containing enough arginine to allow growth of the auxotrophs. Several assays were

conducted by treating the cells in PEG solutions for 30 min, then washing them and plating on minimal agar medium. Positive results were obtained in two experiments and negative results in several others. We finally obtained reproducible positive results by using the following simple method. About $4-7 \times 10^7$ cells of each strain were sampled with a platinum loop and transferred onto a minimal agar medium¹¹ in Petri dishes (9 cm diameter). The cells were mixed with a loop, then 0.1 ml of the following fusion solution was added: 50 mM PEG 6000 Merck, 7.5 mM $CaCl_2$, 5 mM glycine, NaOH to make pH 8.0. This solution was sterilised by filtration through a Millipore membrane (0.45 μ m). The cells were carefully mixed in the fusion solution then spread on the agar surface. Two types of control were made: (1) each strain was separately treated with the fusion solution; and (2) a mixture of the two strains was treated with distilled water. After an eight-day growth at 25°C 8,000 lux, 30–100 colonies ranging between 0.2–1.2 mm diameter were observed in the plates corresponding to the fusion treatment. No colony was observed in the controls.

Four colonies were isolated and subcultured for further analysis on medium deprived of arginine. A microscopical observation of the four isolates revealed the presence of a cell wall in all of them. This observation was confirmed by electron microscopical analysis (Fig. 2). The fusion products form a wall as do the diploids obtained by sexual mating.

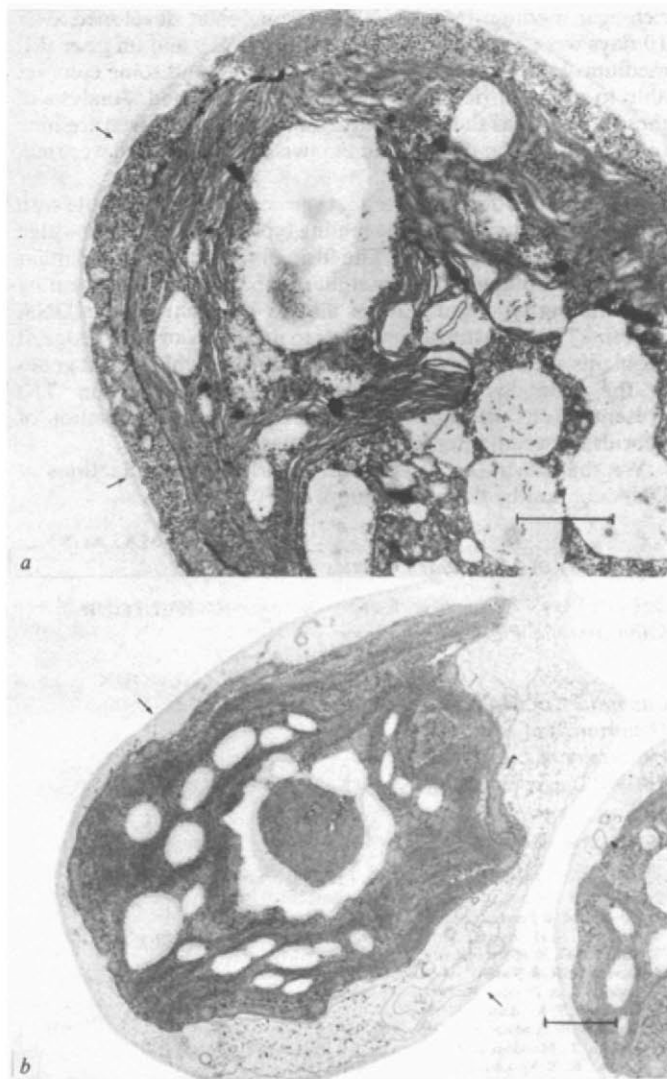


Fig. 2 Ultra-thin sections through cells of a fusion product 303 $\times$ 308/1 (a) and a diploid $arg-7 \times arg-7-3$ (obtained by sexual mating) (b). The cell wall is present (arrow). The samples were prepared for electron microscopy as described in the legend to Fig. 1. Scale bar, 1 μ m.

The fusion products were compared to the wild-type haploid strain and to a diploid strain (*arg-7* × *arg-7-3*) obtained by sexual mating: they were analysed for their cell volume, nuclear volume, total DNA content and chloroplast DNA: total DNA ratio. The results (Table 1) indicate that the fusion products are very similar to diploids as far as cellular volume, nuclear volume and DNA content per cell are concerned. The fusion product 303 × 308/1 (the only one analysed for that criterion) has however a lower chloroplast DNA content (7.3% of total DNA) than that of the haploid or diploid strains (14.1 and 11.1%). Chiang¹⁵ and Lane and Sager¹⁶ found that vegetative haploid cells contain about 14% chloroplast DNA whereas gametes contain only 7%. We do not know whether the low value found for the isolate studied here corresponds to a peculiarity of the strain or if it is a common feature of the fusion products. This point will be further analysed.

The fusion products were, moreover, found to be mating type plus (Table 1): they differ in that respect from diploids obtained by sexual mating which are always mating type minus (*mt*⁻ gene is dominant over *mt*⁺). The four strains were crossed with gametes of haploid wild type *mt*⁻. Zygotes were visualised in all crosses, indicating that the four strains were fertile. The zygotes issued from two crosses were plated on minimal medium containing 4% agar and matured in the dark⁴⁷. The unmated cells were killed by exposure to chloroform vapour and after germination of the zygotes the meiotic products were plated on a rich agar medium (M-N+YE). The colonies developed after 10 days were replicated on rich (M-N+YE) and on poor (M) medium. In both crosses, wild type colonies and some colonies able to grow on rich medium only were detected. Analysis of these auxotrophs showed that they specifically required arginine for growth. This indicates that the two strains analysed were still bearing *arg*⁻ markers.

These results demonstrate that somatic fusion is possible with cell wall mutants of the same mating type (*mt*⁺) in the green alga *Chlamydomonas reinhardtii*. The fusion products are very similar to diploids obtained by sexual mating but differ from them by their mating type and perhaps also by their chloroplast DNA content. They constitute new tools to improve our knowledge of problems such as the maternal inheritance of chloroplast genes or the gene dosage effects in mating-type expression. The present results also open new possibilities for the isolation of hybrids between different algae species.

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Genetic analysis of maternal information in *Drosophila*

EMBRYONIC development is controlled by the information carried by the gametes of the zygote's parents. However, although the chromosomal information is similar in both gametes the oocyte also provides a structural frame (determining embryonic axes), determinants in the cortex and a metabolic pool to support early embryonic events (see ref. 1). The active transcription of thousands of DNA loops in the lampbrush chromosomes of amphibian oocytes suggests that the egg contains transcripts of a large fraction of the genome². In sea urchin oocytes the mRNA complexity might account for the synthesis of several thousands of different protein species^{3,4}. Genetic analysis should help to establish the functional meaning of the information present in the oocytes for embryonic development. In this study we have analysed the effect of chromosome deletions on the development of *Drosophila* embryos. The results indicate that the products of a large proportion of the maternal genome, present in the oocyte, are sufficient and necessary for epidermal morphogenesis and differentiation in the embryo.

In *Drosophila* about 90% of the genes have lethal alleles⁵, and about 30% of those lethals die in the homozygous condition in embryonic stages⁶. The lethal effective phase (LEP) of most of these mutants is late—after completion of morphogenesis and sometimes even after completion of epidermal differentiation⁷. Lethal mutations caused by chromosome deletions may show earlier LEPs, possibly because they have a higher probability of including null-alleles of early acting genes^{7,8}. However, it is not known how far the LEP of the embryos is due to the actual genetic constitution of the zygote or to insufficient supply of gene products from the heterozygous mother to the oocyte.

The chromosome deletions used in this study were previously defined as being homozygous cell lethal in clones of adult epidermal cells resulting from mitotic recombination⁹. This cell lethality implies that products of the genes contained in the deletion are necessary for cell division and/or cuticular differentiation. These deletions might therefore be expected to prevent cell proliferation and cuticular differentiation in homozygous embryos. In this study we analyse the lethality of embryos from parents both of which are heterozygous for chromosome deletions (HM cross) compared with that of embryos from heterozygous mothers but wild-type fathers (♀ HT cross) or from heterozygous fathers but wild-type mothers (♂ HT cross). Whereas the first cross gives rise to homozygous (25%) and heterozygous embryos (50%) the second and third cross yields only heterozygous embryos (50%). As all the deletions studied are recessive lethals, heterozygous embryos are expected to show normal morphogenesis and differentiation.

To avoid lethality due to deleterious factors present in the rest of the genome the experimental crosses were made with F₁ flies from an outcross of the deletion-carrying stock to a wild-type (Vallecas) stock. Females were maintained with an excess of males for 3–4 d in rich medium and afterwards transferred every day to an egg deposition chamber for 3–4 h. Eggs were counted after removal of the parents. After 24 to 26 h (embryonic development takes 21–22 h) the unhatched eggs were counted, dechorionated in sodium hypochlorite (7%) and observed under paraffin oil under a compound microscope. Samples of these eggs were mounted in Faure's medium. We have classified the LEPs of dead embryos as before any visible cuticular differentiation (before stage 9 of Bownes¹⁰) or with clear segmentation and cuticular differentiation of mandibles, tracheae, dorsal and ventral bands of hooks (stage 14). Dead embryos between stages 9 and 14 occurred in less than 10% of all crosses studied. Samples of eggs from each cross were studied 4–6 h after laying to check that all had initiated development. We have made no

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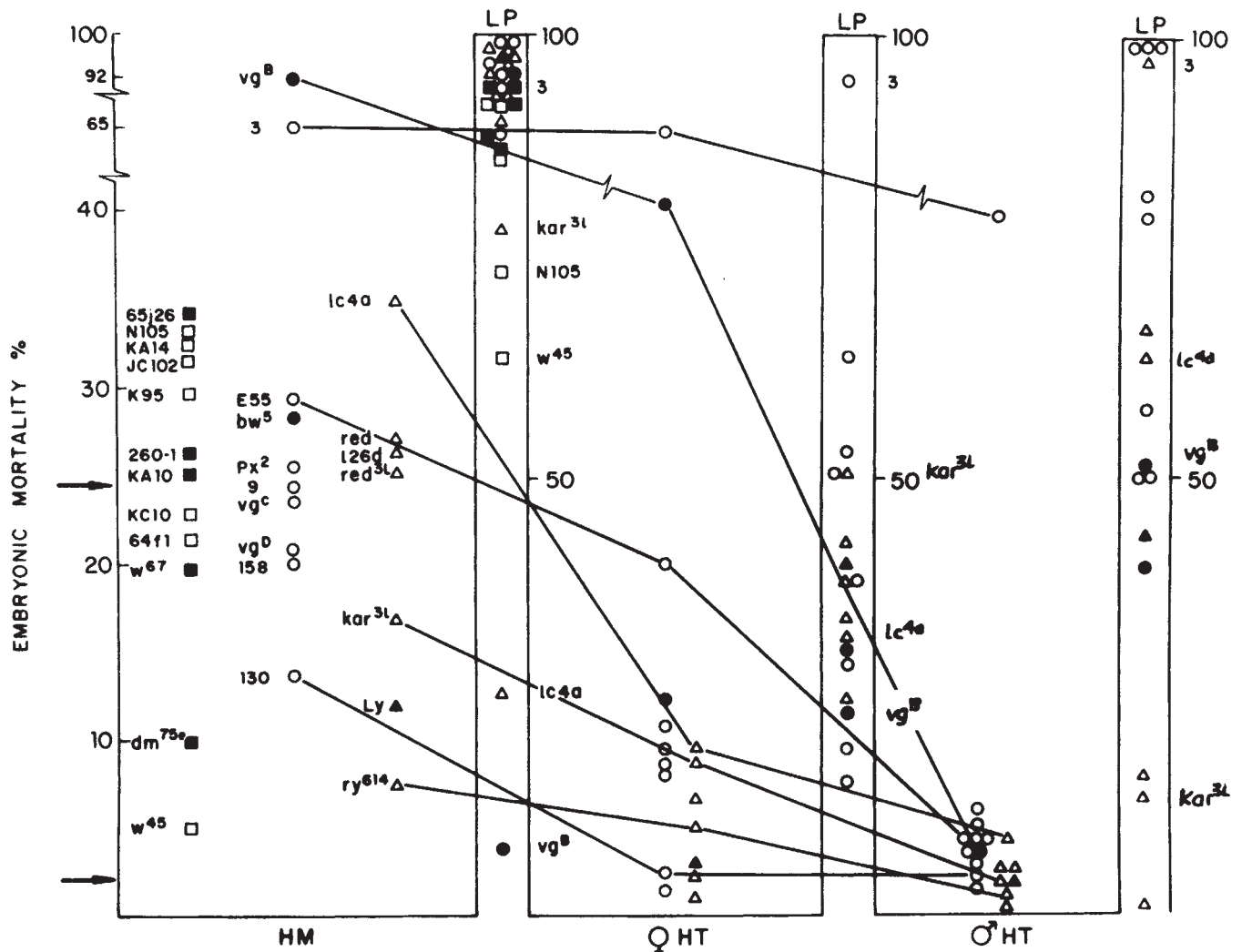


Fig. 1 Per cent of lethal embryos and embryonic lethality phase (LP) in crosses of flies carrying chromosome deletions. LP, percentage of lethal embryos which reach stage 14 (ref. 10). Deletions of X(□), 2nd (○) and 3rd (△) chromosome (deletions described in ref. 9). Open symbols indicate deletions which are not viable in clones of epidermal cells; full symbols viable ones⁹. HM, cross of heterozygous mothers to heterozygous fathers (for deletions in X, males are wild-type). ♀ HT and ♂ HT, crosses where either the female or the male were deletion heterozygotes, crossed to wild-type flies. Percentages of lethal embryos based on 5–10 laying periods and 400–1,200 eggs. Arrows, expected lethal fraction in the HM cross and in wild-type control flies. Connecting lines between representative deletions in the three crosses.

attempt to determine the phenocritical phase⁶ (earliest mutant effects) of these deletions (see below) nor how they affect morphogenesis of internal organs.

For the various crosses the numbers of lethal embryos and their LEPs are presented in Fig. 1. In the HM cross the proportion of lethal embryos varies around the expected 25%. Lower values indicate that some of the homozygous embryos are capable of hatching as larvae and should die in subsequent developmental stages. Higher values indicate that not only homozygous embryos but also other sibling embryos (heterozygotes or even wild type) die. Surprisingly, in most of the deficiencies studied the embryos reach stage 14 (Fig. 1). They show normal cuticular segmentation and differentiation as well as malpighian tubules and even active movements. These findings indicate that gene products which are essential for epidermal cell proliferation and for cuticular differentiation in imaginal disks must either have been available from the oocyte or are not required for embryonic development.

The following data indicate that maternal gene products are necessary for early development. In 8/17 ♀ HT crosses embryonic lethality is significantly higher than in wild-type controls (2%) (Fig. 1). This lethality could result from the genetic insufficiency of deletion heterozygous embryos. However, the lethality found in the ♂ HT cross, which gives rise to the same

heterozygous embryos is, in 8/17 of the deletions studied, lower than in the ♀ HT cross (Fig. 1). Thus, the lethality in embryos from deletion heterozygous mothers must be due to the haplo-insufficiency of the genes included in the deletion chromosome in the oocyte. Lethality phase data for embryos from the ♀ HT cross support that inference: these embryos die mainly in the early stages of development. This suggests that, for some of the zygotes, the chromosomal information is not expressed in time to rescue the oocyte insufficiency. Embryonic lethals usually show several lethal phases⁷, so it is possible that the different LEPs found in embryos of the HM cross actually correspond to the effect of either maternal or zygotic insufficiency.

We have studied 29 deletions in the major chromosome arms, extending from 2 to 30 salivary chromosome bands⁹. The total number of deleted bands studied is 342, of which 283 are not overlapping. The latter figure corresponds to about 6% of the total number of bands (5,000) in the polytene chromosomes. If the chromosome regions analysed are considered as a representative sample of the genome, several general conclusions can be drawn. There is a poor correlation between the size of the chromosome deletions and embryonic lethality in the HM cross ($R=0.35$) or in the ♀ HT cross ($R=0.32$), supporting the conclusion^{7,8} that lethality does not result from additive effects of the deleted genes. More than 50% of the homozygous

embryos of all the deletions studied reach advanced stages of embryogenesis, including normal segmentation and cuticular differentiation. To that group four *minute* loci studied and the *nullo-IV* embryos¹¹ should be added. Thus, for epidermal derivatives it seems that embryonic morphogenesis and differentiation can proceed in the absence of many genes including those necessary for the viability of epidermal cells in imaginal anlagen. Both developing systems, embryo and imaginal anlagen, differ in that whereas the imaginal anlagen undergoes extensive cell proliferation during the larval period, cell proliferation in the embryo following blastoderm is minimal^{12,13}. Thus enough maternal gene products (mRNAs or proteins) may perdure in embryonic cells to allow normal segmentation and cuticular differentiation. If this interpretation is correct then there are two important corollaries.

Lethals with phenocritical phases⁶ in post-embryonic stages or affecting particular tissues cannot be used to indicate the existence of specific genes for larval or imaginal development or specific tissues. It is possible that a large fraction of genes are transcribed in the oocyte and active in embryonic as well as in other developmental stages and in several tissues, but have different perdurance periods. The difficulty of ascertaining phase and tissue specificity of genes is increased when we know only their leaky mutant alleles.

Mutant alleles with phenotypes in the progeny of homozygous mothers (maternal-effect mutants) are known in a small fraction of the genetic loci^{14,15}. Some were shown to be allelic to homozygous visible mutants, others to express the phenotype only at restrictive temperatures. The study of their null-alleles will help to distinguish maternal information genes proper from genes with pleiotropic effects during development and oogenesis.

If embryonic morphogenesis, at least for cuticular segmentation and differentiation, can proceed without the zygotic contribution of an important fraction of the genome, how is it achieved? Systematic study of deletions in lethal embryos may show which genes are responsible for structural elements and which are involved in the specification of segments¹⁶, germ layers and tissues. The haplo-insufficiency shown by some deletions in heterozygous mothers should help to distinguish morphogenetic effects assignable to the female gamete from those which result from the zygotic constitution and only appear in homozygous embryos.

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Meiotic crossing over exchange in the female mouse visualised by BUdR substitution

ALTHOUGH chiasmata have long been thought to be sites of crossing over exchange between chromosomes during meiosis, in keeping with the partial chiasmotype hypothesis^{1,2}, there has been no convincing visual evidence of this despite many attempts (summarised in refs 3-6). Taylor⁷ confirmed by autoradiography that meiotic crossing over occurs by exchange involving breakage and reunion of chromatids. However, because of the poor resolution of autoradiography, requiring study of meiotic chromosomes after 1st metaphase, and because of interference by sister chromatid exchanges, a one-to-one correspondence between chiasmata and exchanges of labelled chromatid segments could not be established^{8,9}. The difficulty was alleviated by correlating chiasmata and exchanges with temperature variation¹⁰, or by selecting a species with localised chiasmata and thus with predictable points of exchange¹¹. 5-Bromodeoxyuridine (BUdR) substitution with differential labelling and consequent differential staining of mitotic chromosomes¹²⁻¹⁵ offer the potential for studying chromosomes at 1st meiotic metaphase, giving an unequivocal picture and separating sister chromatid exchanges from chiasma exchange points between homologues. Tease¹⁶ has reported success with male grasshopper meiotic chromosomes, but an attempt with the male mouse showed only sister chromatid exchanges in the sex chromosomes, and the autosomal bivalents at 1st meiotic metaphase were not differentially stained¹⁷. In the male Armenian hamster the sex chromosomes showed differential staining and an apparent exchange of segments but again the autosomes were not critically differentially stained¹⁸. This may be partly due to the make-up of the male meiotic chromosomes. We have overcome these problems by using an *in vitro/in vivo* technique¹⁹ which enables us to expose the differentiating germ cells of embryonic ovaries to BUdR *in vitro* and to collect and

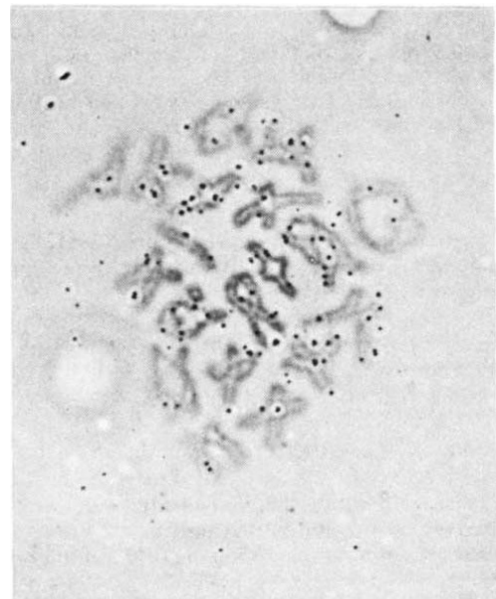


Fig. 1 14-Day (plug = 1) embryonic ovaries were set up in culture on agar strips in Ham F10 (Gibco Biocult) plus penicillin/streptomycin, L-glutamine, 20% calf serum and 3 μ M deoxycytidine, with ³H-BUdR, 0.1 μ Ci ml⁻¹ (0.5 Ci mmol⁻¹). After 48 h they were transferred to basic medium without ³H-BUdR, and transplanted on day 8 of culture (setting up day 0), with minimum light throughout. Meiotic preparations from ova were collected on day 19 (transplant day 0). Autoradiography was carried out after stripping film application and 6 weeks development (photographed Kodak 5460, linear magnification $\times$ 800).

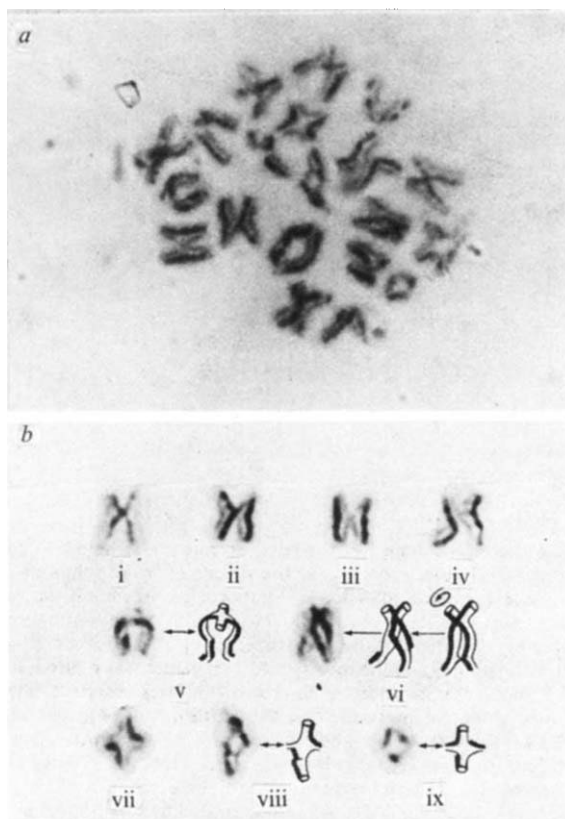


Fig. 2 14-Day embryonic ovaries were cultured in basic medium with cold BUdR, $2.0 \mu\text{g ml}^{-1}$. After 24 h this was changed to basic medium without BUdR. They were transplanted on day 8 and ova were collected on day 19. The cells were stained with Hoechst fluorochrome and examined by differential fluorescence in a UV light microscope. They were then exposed to daylight for 3 d, stained with 10% Giemsa (Gurr) in phosphate buffer, pH 6.8, and mounted in Xam (for details see legend to Fig. 1; linear magnification $\times 800$). *a*, 1st meiotic metaphase with differentially labelled chromatids. *b*, Meiotic configuration selected from *a*, with diagrams (see text). Heavy and light lines reflect the pattern of BUdR substitution; circles represent centromere positions. Note that in *v* an exchange between homologues has occurred very near the centromere.

display the chromosomes²⁰ of the mature oocytes some time after transplantation into young spayed adults. We report our results here.

We used A Strong female fetuses as donors and CBA/H-T6T6 females as recipients, and were completely successful, but in general it is known that even in conditions similar to ours ovarian isografts fare better than allografts²¹. By chase-labelling germ cells *in vitro* with ^3H -thymidine, followed by autoradiographic scoring at pachynema/diplonema 4 d later, we established the optimum time for BUdR labelling in our system and strain.

Using ^3H -BUdR we were able autoradiographically to ascertain its presence in most 1st metaphase chromosomes (Fig. 1). Subsequent use of the minimal concentration of BUdR which gave differential staining of mitotic chromosomes, enabled us to obtain such staining of the meiotic chromosomes in our system. Because of the distinctive differential staining of the chromatids (light chromatids have more BUdR than dark ones) label segregation can provide critical information even at 1st meiotic metaphase (Fig. 2*a*), thus obviating some of the problems with autoradiography. All three types of side-by-side chiasma formation are observed (Fig. 2*b*, dark to dark: i; dark to light: ii, etc.). Two configurations (Fig. 2*b*, v, ix) of the displayed cell show the points of crossing over and one of these also shows an interstitial sister chromatid exchange (Fig. 2*b*, v). In two of the three 'crosses' (Fig. 2*b*, vii, viii, the latter of which is undergoing separation of homologues) the point of exchange corresponding

to the single chiasma is not detectable, and these crosses can be interpreted as being derived from exchanges between equally labelled homologous chromatids (Fig. 2*b*, i or iii and iv). It is also interesting that one of the configurations (Fig. 2*b*, vi) shows a three-strand double involving proximally two dark chromatids and distally a dark and a light one. The latter we interpret as a resolved chiasma; we can therefore detect the point of distal exchange in the two homologous chromatids. In an unlabelled metaphase preparation the latter crossover point would have been undetected.

Our data do not indicate whether or not chiasmata are terminalised in the mouse, or at least in the female of the species. Tease¹⁶ found that there is no terminalisation in the male desert locust. The issue of terminalisation⁵ is relevant both theoretically (assessment of chiasma and crossover frequency) and practically (alleged factor in maternal-age-dependent non-disjunction in mammals²²).

In conclusion, this is the first demonstration of partial chiasmotypy with crossover exchange, and its unequivocal relation to chiasmata, in a female mammal, by an *in vitro/in vivo* technique which is very versatile in the investigation of other problems of mammalian female meiosis.

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Movement of microorganisms in viscous environments

SOME microorganisms swim well in solutions containing viscous agents (molecules with long unbranched chains, such as methylcellulose)¹⁻⁷. *Leptospira*, a slender helical bacterium, swims more rapidly in such an environment than it does in water³, even at viscosities of several hundred centipoise (1 cP = $10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$). This behaviour is baffling until one realises that solutions of viscous agents are highly structured (gel-like). The solute forms a loose quasi-rigid network easily penetrated by particles of microscopic size. The network can exert forces

normal to a segment of the body of a slender cell even when that segment does not possess a component of velocity in the normal direction; hydrodynamic treatments of the motion of micro-organisms (or of cilia and flagella) do not apply. Solutions containing highly branched polymers, for example, Ficoll, are much more homogeneous. Here, we will review existing evidence for the gel-like character of viscous agents and describe experiments in which the motion of *Leptospira* or *Escherichia coli* are compared in solutions of methylcellulose and Ficoll of the same apparent viscosity. Our data show that solutions of methylcellulose are gel-like even when quite dilute, when the bulk viscosity is as small as 2 cP.

The viscosity of a liquid, η , is defined in terms of the force, F , required to maintain a velocity gradient, dv/dx , between adjacent planes of area A ; thus, $F = \eta A dv/dx$. In a true liquid, η is independent of the size of the velocity gradient (of the rate of shear), and F is parallel to v . In a non-newtonian liquid, η depends on the rate of shear; an apparent viscosity is defined as a function of the rate of shear. If the medium is quasi-rigid, F may not be parallel to v .

A macroscopic determination of η can be made by dropping a ball through the liquid and measuring its rate of fall. The ball is subjected to a viscous force, $F = 6\pi\eta av$, where a is its radius and v its velocity (Stoke's law). η can be determined microscopically by using a sphere of microscopic size and measuring its sedimentation rate or, alternatively, its diffusion constant. The diffusion constant, D , and the frictional drag coefficient, $f = F/v$, are related by the Einstein-Smoluchowski relation, $D = kT/f$, where k is Boltzmann's constant and T is the absolute temperature. For a sphere, $D = kT/6\pi\eta a$. For water and aqueous solutions of substances of low molecular weight (MW), measurements of rates of fall, sedimentation rates and diffusion constants all lead to similar values for η , even with particles as small as globular proteins.

The situation is radically different for solutions of unbranched polymers. Their macroscopic viscosities are high, their microscopic viscosities low. A 1% solution can have an apparent viscosity thousands of times greater than that of the solvent (depending on the rate of shear and the ionic strength: the viscosity of solutions of cellulose decrease markedly at high rates of shear⁸; methylcellulose fails to increase the viscosity of 4M NaCl, K. W. Foster, personal communication). However, the sedimentation rates and diffusion constants of small particles change relatively little. Their motion depends more on the viscosity of the solvent than on the viscosity of the solution; the solution acts as a molecular sieve. For example, the diffusion constant of bovine serum albumin in 0.3% (w/v) rooster-comb hyaluronic acid (weight-average MW 1.7×10^6 , a solution of apparent viscosity about 30 times that of the solvent⁹) was found to be only 20% smaller than that in the solvent¹⁰. A similar value was observed in a 1.2% Ionagar gel¹¹. The rotational diffusion constant of serum albumin was not measurably affected by hyaluronic acid¹². In general, the larger the particle or the higher the concentration of viscous agent (or gel), the more restricted the movement^{10,11}. Methylcellulose behaves similarly¹³.

Given this porosity, it is not surprising that a bacterium that can swim in water (by whatever mechanism) can move through a solution of a viscous agent, provided that its body (protoplasmic cylinder) is sufficiently thin. Indeed, some bacteria known to swim in such solutions have been isolated on the basis of their ability to pass through cellulose ester filters (pore size 0.3 or 0.45 μm) and to form spreading subsurface colonies in 1% nutrient agar¹⁴⁻¹⁸. Other bacteria that swim in viscous media, for example, *Leptospira*, have a similar colonial morphology¹⁹. Most of these organisms are helical, but some are not^{4,18}; most are less than 0.4 μm in diameter.

When a helical bacterium swims, it spins about its helical axis. In water, more than one revolution is required to carry the cell forward a distance equal to the pitch: the helix slips circumferentially. In a solution of a viscous agent, on the other hand, the helix moves without slip, like a corkscrew through a cork. *Spirillum volutans* behaves this way in 5% gelatin²⁰. *Spirochaeta*

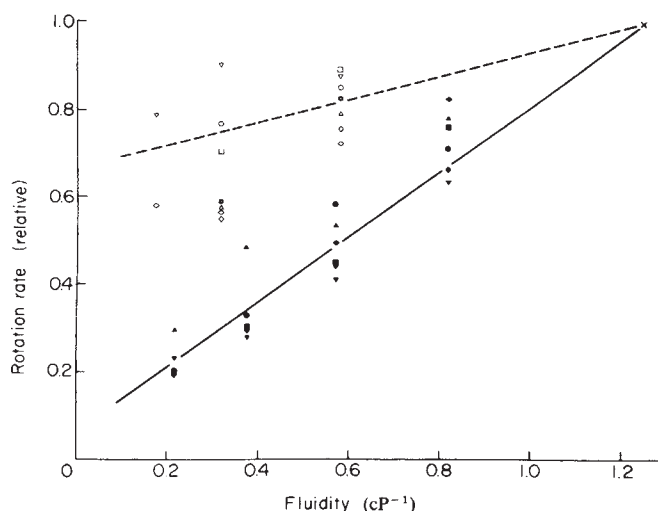


Fig. 1 Relative change in the rotation rates of tethered *E. coli* (strain AW405) as a function of the fluidity ($1/\eta$) of solutions of Ficoll (solid symbols, solid line) or Methocel (open symbols, dashed line). Clockwise and counterclockwise rates were normalised separately (by dividing by the corresponding rate observed in motility medium) and the mean of the two values was plotted; the two values were about the same. The mean rates observed in the absence of the polymer were 8.1 (●), 8.2 (■), 11.2 (◆), 11.4 (▲), 12.9 (▼) and 13.0 (◆) Hz, and 6.8 (○), 6.8 (□), 7.0 (◇), 7.6 (△), 9.4 (▽), 12.6 (◊) and 13.0 (⋈) Hz. The concentrations (% w/v) and bulk viscosities (cP) of the solutions were, respectively: for Ficoll 0, 0.802; 2.5, 1.22; 5.0, 1.76; 7.5, 2.68; 10.0, 4.61; for Methocel 0, 0.802; 0.1, 1.72; 0.2, 3.16; 0.3, 5.74. All measurements were made at 32.0°C. The lines were drawn from the point at fluidity 1.25 through the mean values of the clusters near fluidity 0.6.

aurantia does so in 1% methylcellulose (the helical axes of the best swimmers are straight; our observation on var. *aurantia* strain J1, unpublished), so does *Leptospira interrogans* serotype *illini*²¹ (the helical axis is bent into a spiral at the front of the cell, a hook at the rear; the ends of the cell gyrate, and the protoplasmic cylinder moves without slip along the local helical axis; see also refs 7, 22-24).

This behaviour implies that solutions of viscous agents are quasi-rigid. Circumferential slip is essential if a helical object is to develop thrust in a true liquid, that is, if it is to propel itself using viscous forces; hydrodynamics demands it^{25,26}. However, it is not required in a rigid environment, that is, in one that can push on an object in a direction normal to its surface. Gray illustrated this point in a discussion of undulatory propulsion by comparing the waving motion of the tail of a spermatozoon to the serpentine movement of a snake²⁵. The resistances offered by the media through which (or over which) these organisms move (water for the spermatozoon, stones for the snake) are fundamentally different.

The helical bacteria act like passive rigid screws driven by an external torque. In *Spirillum* the torque is provided by the rotation of the polar flagella²⁰, in *Spirochaeta* by the roll (or flow) of the external sheath²⁷, and in *Leptospira* by the gyrations of the ends of the cell²¹. In a gel-like medium, the cells thread or force their way through the spaces between the molecules of solute. *Leptospira* swims efficiently in such an environment³; the gyrations of the ends of the cell are hindered, and the roll of the protoplasmic cylinder is enhanced²¹.

Leptospira behaves in a different manner in solutions of methylcellulose or Ficoll of the same apparent viscosity. It does not swim efficiently in solutions of Ficoll, which are less structured. Methylcellulose (Methocel 90 HG, 4,000 cP, Dow, a hydroxypropyl methylcellulose product) is an unbranched polymer of MW 143,000; its solutions are newtonian at concentrations of $\leq 0.5\%$ (w/v), non-newtonian at higher concentrations²⁸. A stock solution (1% w/v) was prepared in a motility medium containing 0.067 M NaCl, 0.01 M potassium phosphate (pH 7.0) and 10^{-4} M EDTA (by stirring overnight at room

temperature, 21 °C). Solutions at lower concentrations were prepared by dilution in the same medium. Ficoll (Ficoll 400, Pharmacia) is a highly branched polymer of sucrose and epichlorohydrin of MW ~400,000; its molecules are roughly spherical²⁹, its solutions newtonian³⁰. Solutions of various concentrations were prepared directly in motility medium (by stirring at room temperature). All solutions used in the experiments (of both Methocel and Ficoll) were sterilised by passage through cellulose ester filters (Millipore, pore size 0.45 µm) and stored at room temperature. Their viscosities were determined in Cannon-Ubbelohde viscometers (at the temperatures specified; flow times 1–6 min). *Leptospira interrogans* serotype *illini* was grown at 30 °C on a Tween-80 albumin medium³¹ (less glycerol) to an optical density of about 0.3 (by eye). Samples of this suspension (0.02 ml) were mixed with aliquots of solutions of Methocel or Ficoll (1 ml) and examined by dark-field microscopy (at room temperature, between a coverslip and a slide separated by a ring of Apiezon L with a Nikon S-Ke microscope: 30 W tungsten or 100 W tungsten-halogen lamp, ultra dark-field condenser, Fl 40× plan objective, 15× oculars).

Serotype *illini* translated rapidly in 1% Methocel ($\eta = 95$ cP at 32 °C, our measurements; 140 cP at 25 °C and zero rate of shear, ref. 28). It swam in a serpentine fashion in the hook-spiral configuration, spiral-end first, the protoplasmic cylinder moving without slip^{21–24}. Cells with only one hook (or spiral) translated at about the same speed, spiral-end first or hook-end last²¹. Translational velocities of more than $20 \mu\text{m s}^{-1}$ have been reported for other strains in similar media³. In contrast, serotype *illini* failed to translate in 35% Ficoll (92 cP at 21 °C). The ends of the cell gyrated slowly; the body remained nearly stationary. The cells had a tendency to wind up on one another.

Differences between the motion of serotype *illini* in solutions of Methocel or Ficoll were still marked at concentrations at which both media are thought to be newtonian^{28,30}. The cells translated well in 0.25% Methocel (4.3 cP at 32 °C) but not as well as in 1% Methocel. Some slip of the protoplasmic cylinder was apparent. Cells with only one hook (or spiral) moved more rapidly spiral-end first than hook-end last. In contrast, the motion in 10% Ficoll (4.6 cP at 32 °C) was hard to distinguish from that in motility medium. The cells gyrated and rolled rapidly but translated slowly. Cells with only one hook (or spiral) moved spiral-end first but not hook-end last²¹.

A more quantitative idea of the difference in behaviour between solutions of Methocel and Ficoll was obtained by comparing the rotation rates of tethered *E. coli* in the two media. Cells were grown and tethered as described previously³² and observed at 32 °C, either with the tracking microscope³³ or with a version of the linear-graded filter apparatus³⁴ in which an image from the microscope is analysed directly. The same cell was studied in Ficoll or Methocel at various concentrations. With Ficoll the rotation rates were nearly inversely proportional to the viscosity, as expected if the flagellar motors run at a constant torque; with Methocel they were not (Fig. 1). Solutions of Methocel perturbed the motion of *E. coli* less than did solutions of Ficoll of the same apparent viscosity; evidently, the cells were able to push the chains of methylcellulose out of the way and to move more as they would in pure solvent. Note that all the solutions used in these experiments were passed through Millipore filters (see above); they were homogeneous both by eye and by phase-contrast microscopy. However, differences were apparent to *E. coli*, even at the smallest concentrations tested (Ficoll 2.5% w/v, Methocel 0.1% w/v).

Many kinds of bacteria swim more rapidly in dilute solutions of viscous agents (viscosities of the order of 2 cP) than they do in ordinary media^{1,2,6}. The efficiency of flagellar propulsion is probably enhanced by the gel-like structure of these solutions. Dextran, a branched polysaccharide, failed to show the effect¹; Ficoll has not been tested. Flagellar bundles are more easily seen in solutions of viscous agents than in ordinary media^{35–38}. Is this also due to a qualitative change in the structure of the medium? Does Ficoll show this effect? Is there a better agent that could be used for experiments of this kind? The choice is limited, as most

bacteria fail to swim in solutions of high ionic strength or high osmolarity. If one's purpose is to slow the motion of a micro-organism (or a cilium or flagellum) while keeping the hydrodynamics the same, Ficoll is a better choice than methylcellulose or probably any other unbranched polymer.

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Growth of human B-lymphocyte colonies *in vitro*

RECENTLY developed *in vitro* culture systems that support clonal formation of lymphoid cells have promoted remarkable advances in our understanding of lymphocyte proliferation and the nature of the proliferating cells. Human T lymphocytes from venous blood, bone marrow and lymphoid organs and murine T and B lymphocytes from lymphoid tissues have been grown into colonies of T and B cells in semisolid culture. In these systems, T-lymphocyte colony formation requires that the seeded cells undergo T-mitogen stimulation, that is, by phytohaemagglutinin (PHA) or concanavalin A, and B-lymphocyte colony growth necessitates sensitisation by mercaptoethanol, a humoral thymic factor, or a polyclonal B-cell activator, such as lipopolysaccharide, purified protein derivative or dextran sulphate^{1–7,17}. Previous attempts to achieve colony formation of human B cells were unsuccessful, even in the presence of known polyclonal B-cell activators. However, in certain experimental conditions, the plant lectins, pokeweed mitogen (PWM) and PHA, stimulate both human B and T lymphocytes into transformation and

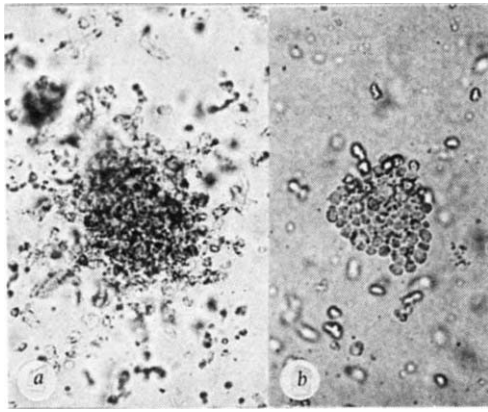


Fig. 1 Viewed under a light microscope at low magnification, unstained colonies grown in agar medium from human, venous blood, B-lymphocyte-enriched population stimulated with PHA. *a*, Large colonies (type I) containing 250–500 cells which developed within the upper agar layer 5 days after seeding; *b*, small, flat colonies (type II) of 50–100 cells which grew on the surface of the upper agar layer 7 days after plating.

proliferation in cell culture with a synergistic effect between the sensitised T and B cells^{8–13}. We report here that, based on this information and earlier techniques for clonal formation of human lymphocytes *in vitro*, we have evolved a two-layer soft agar culture technique in which the mitogens, PHA and PWM, were able to generate the appropriate signal for triggering B-lymphocyte precursors of venous and umbilical cord blood, tonsil, adenoid and chronic lymphatic leukaemia (CLL) origin into colony-forming and immunoglobulin-producing cells.

Lymphoid cells, obtained from human adenoids and tonsils excised in sterile conditions, were suspended in Dulbecco's modified Eagle's medium (MEM). The cells were incubated for 20 min at 37°C in MEM containing 50 µg ml⁻¹ erythromycin and 200 µg ml⁻¹ kanamycin, then washed twice and resuspended in MEM. Heparinised blood (10 IU ml⁻¹) was obtained from umbilical cord, healthy adult donors and patients in whom

CLL had been diagnosed but who had not received therapeutic treatment. Lymphocytes were isolated by Lymphoprep (sodium metrizoate/Ficoll) gradient (D 1.077)¹⁴, washed twice with MEM containing penicillin and streptomycin (100 U ml⁻¹ and 100 µg ml⁻¹, respectively) and resuspended at 10⁶ cells per ml of the same medium supplemented with 12.5% of inactivated pooled human serum.

B-lymphocyte-enriched populations were obtained from mononuclear cells by separating E-rosette-forming cells by density sedimentation through Lymphoprep. The T-cell-depleted interphase fraction contained at least 70% B cells when normal blood was processed, 65% B cells for cord blood, 70% for tonsils and 80% for CLL. B cells were quantitated by direct immunofluorescent staining of surface membrane immunoglobulins (SmIg) with fluorescein isothiocyanate-conjugated anti-µ chain or polyvalent rabbit antiserum to human immunoglobulins¹⁵. The B-cell-enriched population contained <4% E-rosette-forming cells. For sensitising the cells with mitogen in liquid phase, suspensions of B-cell-enriched populations or mononuclear cells (10⁶ cells per ml MEM containing 12.5% human serum) were incubated with PHA-M (Difco, 0.0125 ml ml⁻¹) or PWM (Sigma 2.5 µg ml⁻¹) for 72 h at 37°C in a water-saturated atmosphere of 7.5–9% CO₂ in air (Table 1). The sensitised cells were then washed, resuspended and seeded in the upper soft agar layer (0.32% agar), at 5 × 10⁵ cells per

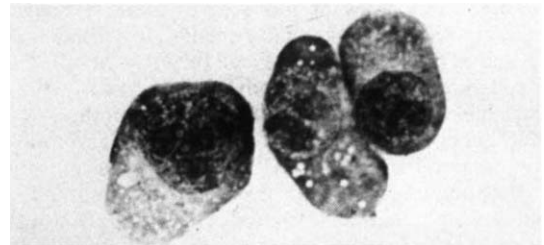


Fig. 3 Colony cells from PWM-stimulated, cord blood B-cell-enriched population, 6 days after plating, stained with May-Grünwald Giemsa. All cells had the morphological features of transformed lymphoblastoid and small or large plasmoid cells.

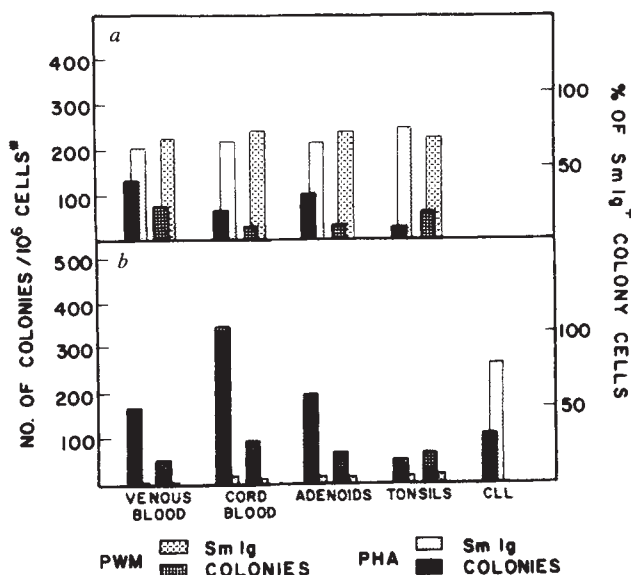


Fig. 2 Lymphocyte colony number and their cell SmIg, grown in Bacto agar from mitogen-stimulated lymphoid cells. All B-cell-enriched populations and CLL cells (80% B lymphocytes) developed into B-cell colonies with a characteristically high content of SmIg⁺ colony cells. Colonies grown from unfractionated lymphocytes from normal human venous blood were uniformly SmIg⁻ and those stemming from normal human lymphoid tissue had few SmIg⁺ cells. The data represent the average of 3 or more experiments (2–4 plates per culture). *a*, B-cell-enriched population; *b*, mononuclear cells.

30-mm Petri dish. The lower agar layer (0.5% agar) contained either 0.0125 ml PHA per ml medium or 2.5 µg PWM per ml medium. The Petri dishes were incubated at 37°C in a fully humidified atmosphere at 7.5–9% CO₂ in air. In some experiments cell-free lymphocyte culture fluid (LCF) obtained from a 3-day culture of cord blood lymphocytes (1.5 × 10⁶ cells per ml MEM containing PHA 0.0125 ml ml⁻¹ or PWM 2.5 µg ml⁻¹) replaced the MEM of the lower agar layer¹⁶. For controls we used cultures which did not contain mitogen in the lower agar layer or in which the lymphocytes seeded in the upper layer had not been presensitised with mitogen.

The formation of the colonies and their morphology were observed under an inverted microscope. Colonies of 50 or more cells developed from PHA-stimulated cells 3–5 days after seeding and from PWM-treated cells after 5–7 days. Seeded PWM-sensitised, B-cell-enriched populations proliferated and formed lymphocyte colonies only when the soft agar culture had been supplemented with LCF. Two types of colonies formed: type I, large colonies containing 50–500 cells appeared within the upper layer, and type II, small, flat colonies containing 50–150 cells developed above the primary colonies, on the surface of the upper agar layer (Fig. 1). A stationary period of 2–3 days (PHA) or 4–5 days (PWM) followed colony development; the colonies began to degenerate on days 7–9, with cell disintegration and lysis. As distinct from the large primary colonies, the number of flat colonies varied greatly. We decided to use the score of the large primary colonies as a parameter for evaluating the effect of stimulation and growth conditions on lymphoid cells. When cord blood cells were seeded, a new kind of colony, composed of small macrophages, formed 12–13 days after culture.

To characterise the nature of the colony cells, 4–6-day colonies were collected, and their cells pooled and washed.

Table 1 Surface markers on mononuclear cells and B-cell-enriched populations before and after mitogen sensitisation

Source of cells	Cell population	% E rosettes	Liquid phase		
			0 h %SmIg ⁺	72 h, PHA* %SmLg ⁺	72 h, PWM* %SmLg ⁺
Peripheral blood	B cell	3.3±0.6	71.7±0.9	73.3±6.6	71.7±0.9
	Mononuclear	75.0±4.1	10.7±0.4	10.7±4.1	12.0±4.2
Cord blood	B cell	3.7±1.1	65.0±4.1	73.0±8.7	77.3±6.2
	Mononuclear	45.0±4.1	11.3±1.3	11.0±2.9	15.3±1.1
Adenoids	B cell	4.0±0.5	71.5±1.5	59.0±9.0	66.0±1.0
	Mononuclear	60.0±0.5	32.5±2.5	21.0±1.0	24.0±0.0
Tonsil	B cell	3.5±0.2	75.0±3.0	68.0±4.0	67.0±3.0
	Mononuclear	39.0±1.0	50.0±0.0	22.5±10.5	25.0±5.0
Peripheral blood (CLL)	Mononuclear	9.7±5.3	79.7±1.3	86.0±2.9	

'B cell' refers to the B-lymphocyte-enriched population, 'Mononuclear' to unfractionated mononuclear cells. It was found that in mitogen-sensitised lymphocyte populations, spontaneous E-rosette formation was no longer specific for T cells.

*After 72 h stimulation with mitogen, the cells were plated in the two-layer soft agar system.

After agar residues had been removed by passing the cell suspension several times through a tuberculin syringe and needle, SmIg were assayed. Detection of SmIg was used as the membrane marker for characterising colony cells. B-cell-enriched populations gave rise to B-cell colonies, as manifested by the large number of colony cells having SmIg⁺, and mononuclear cells evolved T-cell colonies, with SmIg⁻ colony cells (Fig. 2). Also, B-lymphocyte colony growth was consistently obtained from blood and lymphoid tissue B-cell-enriched populations sensitised with PHA or PWM and from CLL (80% B cells) treated with PHA. Mononuclear cells from normal peripheral blood gave rise to colonies whose cells bore no SmIg; among those from cord blood or lymphoid tissue, where the ratio of B to T cells is high, 1–4% SmIg⁺ cells were seen.

Morphological examination of the large B-cell colonies that developed from PHA-induced cells showed that these had compact centres whereas those that grew from PWM-treated cells had a loose, diffuse structure. From a microscopic study of colony cells, we found that both type I and type II colonies contained many ellipsoid or spherically shaped, large mononuclear cells with a blast-like appearance, that is, a round nucleus and basophilic cytoplasm, and plasmoid cells having the

characteristic eccentric nucleus and bulky basophilic cytoplasm (Fig. 3). In CLL colonies, many cells resembling small or medium-sized lymphocytes were also seen. The progeny cells generated by PWM-stimulated B lymphocytes were more differentiated than those of the PHA-treated cells. Electron microscope studies of the cells in B colonies that developed from PHA- and PWM-stimulated populations showed that these had the morphological features of either blast-like or plasmoid cells. Many strands of well developed rough-surfaced endoplasmic reticulum with cisternal dilations were observed, particularly in the PWM-treated cells (Fig. 4).

Differences in the number and morphology of PHA- and PWM-induced colonies and their component cells, dissimilar colony development times and distinct culture conditions (the need for lymphocyte culture fluid for colony formation of PWM-induced cells) indicate that the two mitogens may act either on different B-cell subpopulations or on a B-cell population at different stages of maturation.

This technique for cloning B cells from normal and leukaemic B lymphocytes in soft agar conditions should be a major breakthrough in investigations concerned with the mechanism of the immune response of normal and leukaemic lymphoid populations. It should be particularly helpful for analysing functional B-cell populations, and the role of monocytes and T cells in the activation of B lymphocytes and regulation of their colony growth.

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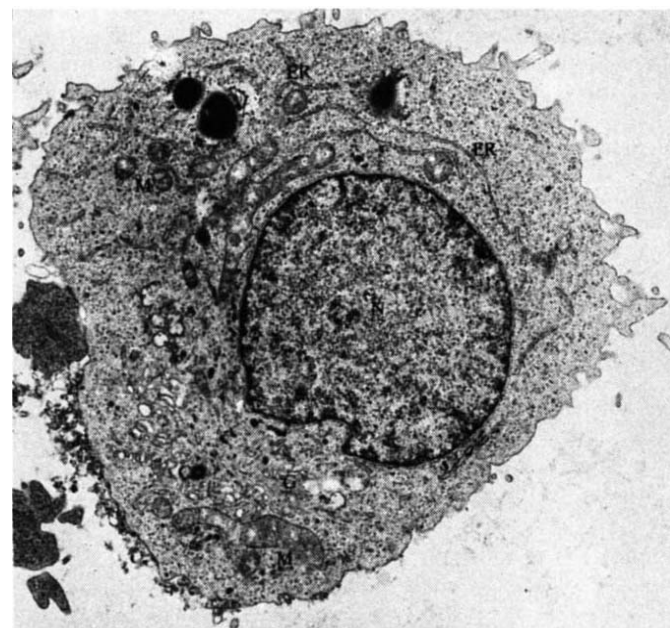


Fig. 4 Electron micrograph of a cell from a colony developed from PHA-sensitized normal venous blood, B-cell-enriched population 4 days after plating. Note that the eccentric nucleus (N) contains increased euchromatin. In the cytoplasm there are large and short rough-surfaced endoplasmic reticulum profiles (ER), many polyribosomes, a centriole (C), the Golgi apparatus (G) and different-sized mitochondria (M). ($\times 7,000$.)

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Cooperation between antigen-reactive T cells and anti-idiotypic B cells in the anti-idiotypic response to antigen-antibody complexes

THE variable regions of antibody molecules carry unique antigenic determinants (idiotypes), which have been shown to be isoimmunogenic, that is, immunogenic in syngeneic animals¹⁻³. Jerne and others^{4,5} have proposed that autoimmune responses to self-idiotypes might form the basis of an immunoregulatory network, and evidence in favour of this hypothesis has been reported from several laboratories⁶⁻⁸. Preformed antigen-antibody complexes of dinitrophenylated keyhole limpet haemocyanin (DNP-KLH) and the DNP-binding myeloma protein M315 are very effective in inducing iso-anti-idiotypic antibodies to the M315 idiotypic in BALB/c mice⁹, and formation of immune complexes during an antibody response could therefore represent the mechanism whereby self-idiotypes become autoimmunogenic. As such anti-idiotypic antibody responses are T-cell-dependent, one must assume that if animals are immunised with purified antibody then anti-idiotypic T (helper) cells cooperate with anti-idiotypic B cells. The existence of iso-anti-idiotypic T cells has been formally demonstrated^{10,11}. When an antibody is administered as an immune complex, a similar cooperative event may occur; but it is also possible that antigen-specific T cells cooperate with idiotypic-specific B cells through the immune complex, as the latter can prime animals for immunity to the antigen¹² and to idiotypic determinants on the antibody (see below). Here I present evidence that this occurs.

Table 1 Cooperation between KLH-primed spleen cells and anti-M315 B cells

Group*	B cells primed with†:	KLH-primed spleen cells‡	Boost §	IBC ($\mu\text{g ml}^{-1}$)¶
1	DNP-BSA-M315	+	DNP-KLH-M315	43.0 ± 11
2	DNP-BSA-M315	+	M315	0.4 ± 0.2
3	DNP-BSA-M315	-	DNP-KLH-M315	2.4 ± 1.0
4	Nothing	+	DNP-KLH-M315	1.7 ± 0.5

*Irradiated (500 R) BALB/c mice ($n = 4$).

†Spleen cells from BALB/c mice given 10 μg (wt of DNP₅₀BSA) DNP₅₀BSA-M315 (1:10 w/w ratio) complexes 3 weeks previously. These cells were treated with anti-Thy 1 antiserum and rabbit complement (45 min for 37°C) and each mouse received 20×10^6 viable cells.

‡ 20×10^6 spleen cells from mice given 100 μg alum-precipitated KLH plus 2×10^9 *Bordetella pertussis* organisms 1 month previously.

§10 μg DNP-KLH-M315 complexes (1:5 w/w ratio) or 50 μg M315 given intravenously.

¶Idiotypic-binding-capacity (determined by double antibody radioimmunoassay using ¹²⁵I-labelled M315 (refs 9, 16) of sera taken 10 d post-transfer.

The first indication for this mechanism emerged from an experiment where mice were immunised with DNP-KLH-M315 immune precipitates, as described previously⁹. Three weeks later, some were boosted with M315 and others with complexes. Those boosted with M315 had similar anti-M315 antibody titres on day 31 to unboosted mice, whereas those boosted with DNP-KLH-M315 gave a true secondary response (data not shown).

In the experiment shown in Fig. 1 BALB/c mice were given DNP-KLH-M315 complexes at w/w ratios of antigen:antibody of 1:10 (antibody excess) or 1:1 (antigen excess)⁹, with or without alum-precipitated carrier, that is, KLH. Administration of KLH increased anti-M315 antibody titres, but only after boosting with immune complexes. The effect was most marked after an optimal (10 μg DNP-KLH) priming dose of DNP-KLH-M315 (1:10) complexes (fourfold increase in antibody),

and less with a 1- μg dose of complexes. Complexes prepared at a 1:1 ratio elicit poor anti-M315 antibody titres, and KLH priming did not enhance their immunogenicity. Priming with KLH also enhanced the anti-DNP antibody response in those mice given 10 μg DNP-KLH-M315 (1:10) (data not shown). Complexes in antigen excess and in antibody excess both induced anti-DNP antibodies (not shown), but only those in antibody excess induced substantial anti-M315 antibody titres.

The helper effect of KLH priming could have been due to the induction of KLH-specific T-helper cells, or to the enhanced production of anti-KLH (or anti-DNP) antibodies¹³. In subsequent experiments groups of mice were immunised with 1:10 complexes of DNP-bovine serum albumin (DNP-BSA) and M315. Three weeks later B cells from these mice were transferred with spleen cells from KLH-immunised mice (as a source of T-helper cells) to irradiated recipients, which were boosted with either DNP-KLH-M315 or M315 (Table 1). Mice given DNP-BSA-M315-primed B cells plus KLH-primed cells gave a marked anti-M315 response, but only when boosted with DNP-KLH-M315, and not when boosted with M315. DNP-BSA-M315-primed B cells alone gave a very weak response, comparable to that given by unprimed B cells plus KLH-primed cells.

In a further experiment irradiated mice were given B cells from mice primed with purified M315 in adjuvant³, with or without purified KLH-primed T cells (Table 2). Again, those given helper cells produced substantial anti-M315 antibody titres, but only when boosted with DNP-KLH-M315 complexes. The idiotype specificity of the cooperation is indicated by the low antibody titres of the group boosted with DNP-KLH-M460 complexes. M460 is a DNP-binding IgA myeloma protein with a different idiotypic from M315. The binding of the antibodies in group 1 to M315 was almost completely inhibited by 10^{-3}M DNP-lysine (not shown), indicating that they were primarily directed against the combining site of the antibody.

These experiments therefore suggest that in mice immunised with antigen-antibody complexes, antigen-specific helper cells can cooperate with anti-idiotypic B cells to generate an anti-idiotypic antibody response. Indeed, the available data suggest that this form of cooperation is the most important in anti-idiotypic immunity to immune complexes. It is unlikely that KLH priming induces anti-idiotypic T cells, since mice do not respond to purified M315 when given KLH-primed T cells and M315-primed B cells (Tables 1, 2). In addition, T cells from mice

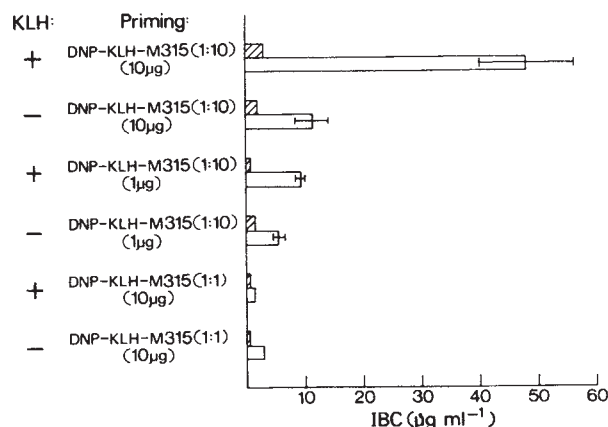


Fig. 1 Effects of carrier (KLH) priming on the anti-idiotypic response to DNP-KLH-M315 complexes. Groups ($n = 4$) of BALB/c mice were given 10 or 1 μg (wt of DNP-KLH) DNP₁₇₂KLH-M315 complexes (w/w antigen: antibody ratios 1:10 or 1:1 (ref. 9) intravenously on day 0. Some also received 100 μg alum-precipitated KLH intraperitoneally on the same day. The mice were bled on day 21 (■), boosted with 10 μg DNP-KLH-M315 and finally bled again on day 32 (□). Anti-M315 antibody titres were determined by double-antibody radioimmunoassay^{9,16}. IBC, idiotype-binding capacity.

Table 2 Cooperation between KLH-primed T cells and anti-M315 B cells

Group*	T cells primed with:†	Boost‡	IBC ($\mu\text{g ml}^{-1}$)§
Exp a			
1	KLH	DNP-KLH-M315	89.2 $\pm$ 3.0
2	KLH	M315	3.5 $\pm$ 1.0
3	KLH	DNP-KLH-M460	2.4 $\pm$ 0.7
4	None	DNP-KLH-M315	3.9 $\pm$ 0.9
Exp b			
1	KLH	DNP-KLH-M315	15.3 $\pm$ 1.6
2	BGG	DNP-KLH-M315	5.0 $\pm$ 2.3
3	None	DNP-KLH-M315	2.5 $\pm$ 0.5

*Irradiated (500 R) BALB/c mice ($n=4$); all received 10^7 B cells (treated as in Table 1) from mice primed with 100 μg M315 in Freund's adjuvant, followed by boosts of M315 in incomplete adjuvant and saline³.

† 10×10^6 nylon wool purified T cells¹⁵ from mice given 100 μg alum-precipitated bovine gamma globulin (BGG) or KLH plus *B. pertussis* 3 weeks previously.

‡10 μg DNP-KLH-M315 or DNP-KLH-M460 complexes (1:5 w/w ratios) or 50 μg M315.

§Idiotypic-binding capacity determined on sera taken 10 d post-transfer.

given bovine gamma globulin do not provide help in this system, thus confirming the antigen specificity of the helper effect (Table 2).

The results shown in Fig. 1 suggest that in the conditions used here DNP-KLH-M315 complexes do not prime KLH-specific T-helper cells optimally (see also ref. 12). Perhaps antigen-antibody complexes do not prime anti-idiotypic T-helper cells efficiently either, or alternatively they may induce anti-idiotypic suppressor cells. The co-existence of anti-DNP and anti-idiotypic antibodies in mice immunised with DNP-KLH-M315 complexes suggests that the M315 idio type is not a prominent component of the anti-DNP antibody repertoire of the BALB/c mouse. As there is evidence that these mice do make the M315 idio type in response to DNP¹⁴, another possibility is that administration of M315 complexes suppresses the expansion of the B-cell clone making this idio type.

In the context of network theories of immunoregulation⁴, the present results suggest that if the generation of immune complexes during an antibody response represents the physiological mechanism for inducing autoimmunity to idiotypes, then these complexes need only prime anti-idiotypic B cells to give an anti-antibody response. These cells could then cooperate with pre-existing antigen-specific T-helper cells, thereby bypassing the requirement for anti-idiotypic T-helper cells.

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Micropuncture of lateral intercellular spaces of *Necturus* gallbladder to determine space fluid K^+ concentration

EPITHELIA such as that of *Necturus* gallbladder consist of a pavement-like sheet of uniform cuboidal cells of $20 \times 20 \times 20 \mu\text{m}$. As seen in the electron microscope, the clefts between the cells (lateral intercellular spaces, LIS) are ~ 0.1 – $1 \mu\text{m}$ wide. They are closed against the gallbladder lumen by so-called 'tight' junctions—which, however, allow the passage of small ions¹—and at the other end communicate more freely with the interstitial fluid compartment of the body through slits of > 30 – 50 nm width. The lateral membranes of the epithelial cells which face the LIS are highly folded (the surface amplification factor in *Necturus* gallbladder is around 20 (ref. 2) but the basal membranes which face the interstitial fluid compartment directly are not, and so it is evident that virtually all matter which is reabsorbed from the lumen after passing the cell membranes (or the tight junctions) must enter the LIS before it reaches the interstitial fluid compartment proper. It is generally assumed that the LIS have a central role in isotonic fluid transporting epithelia by acting as the main coupling site between salt and water transport. Although elaborate models have been used in attempts to describe the salt and water coupling in detail mathematically^{3–9}, the results have been inconclusive, mainly because there was no definitive experimental proof. A central question, whether or not the LIS fluid is hypertonic as postulated by the standing gradient hypothesis of isotonic fluid absorption³, remained open, until recently, when the first direct measurements of the ion concentrations in the lateral spaces were published¹⁰. These data were obtained by means of electron-probe analysis of frozen tissue sections of rabbit ileum and revealed a substantial hypertonicity. We have tried the alternative approach of measuring the ion concentrations in the LIS directly using ion-selective microelectrodes. Our observations, which are reported here, indicate that as far as the K^+ concentration is concerned the ion accumulation in the LIS of *Necturus* gallbladder is much lower than suggested by the electron-probe data from rabbit ileum.

We chose an *in vitro* preparation of *Necturus* gallbladder¹¹ in which we can recognise the LIS under $\times 640$ magnification with

Table 1 Results from measurements with double-barrelled K^+ -selective electrodes

Potential (mV)	f (%)	c_K (mmol l^{-1})	Localisation	Depth of tip penetration
+0.4	71	3.4	Colour	
–1.9	89	4.6	Colour	
–0.9	97	3.2	Colour	
+1	100	3.0	Colour	
+1.5	100	2.8	Profile	
–0.5	96	4.9	Mark	50–75%
+0.2	95	4.4	Mark	50–75%
–2	77	4.6	Mark	~50%
–0.28 $\pm$ 1.28		3.9 $\pm$ 0.8		

'Potential' is the LIS potential referred to subepithelial space. f is the fraction of the overall transepithelial resistance which was observed in the LIS; low values of f indicate the presence of some leak between gallbladder lumen and LIS along the electrode path. c_K is the K^+ concentration of LIS fluid. It was measured by filling one electrode barrel with Corning no. 477317 potassium exchanger as described previously¹² and the other barrel with Ringer solution, recording the potential difference between both barrels and reading c_K from a calibration curve¹². 'Localisation' gives the means of localisation. Because bevelling of double-barrelled electrodes resulted in tip diameters of 1.2– $2 \mu\text{m}$, the last experiments were carried out with unbevelled tips of $< 0.5 \mu\text{m}$ diameter, which did not allow colour ejection. Hence, localisation was achieved by recording the potential profile and the advance of a dye mark on the electrode.

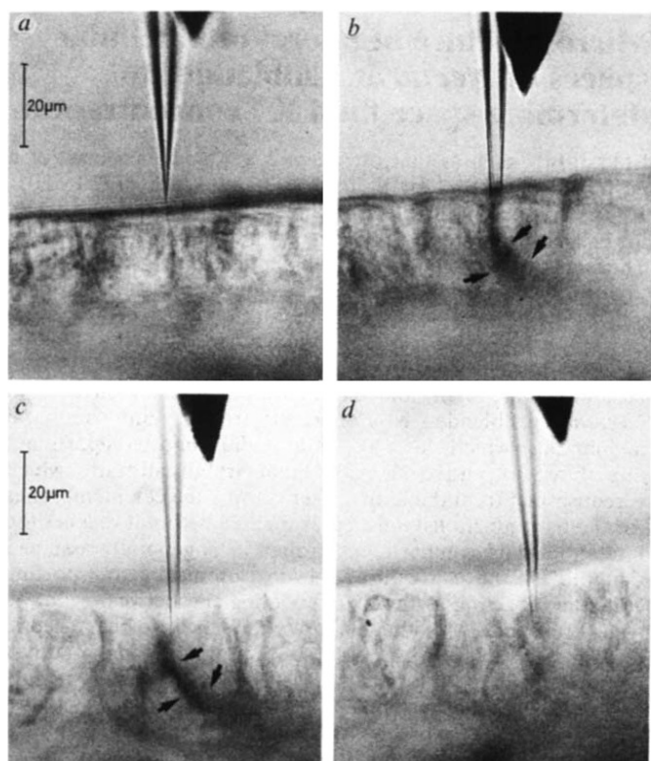


Fig. 1 Photomicrographs of fluid ejection into lateral intercellular spaces. The electrodes were filled with Ringer's solution containing in mmol l^{-1} : NaCl, 110; KCl, 2.5; CaCl_2 , 1; NaHCO_3 , 10, to which 1% of Kiton blue was added. After bevelling of the tip to 0.8–1.2 μm o.d. or, respectively, 70–95 M Ω resistance, enough colour could be ejected to give a clear indication of the position of the electrode tip. *a*, Electrode and epithelium before puncture; *b*, after puncture and during fluid ejection at slightly shifted focus. Between the arrows, the colour can be seen to fill a LIS, although the contrast in the black and white reproduction is poor. *c*, A similar picture of another LIS; *d*, the same place after cessation of fluid ejection and after the electrode has been moved slightly laterally.

a water immersion lens. The bladder was mounted between two half-chambers and bathed on either side with Ringer's solution. We first tried to define criteria for successful impalements of the LIS and measured the electrical parameters of the LIS. Glass micropipette electrodes were mounted on a Leitz micro-manipulator and manually advanced from the lumen compartment into a cell and then further in the direction of a LIS. Because the electrode tip is not visible inside the tissue and because the tissue is usually slightly squeezed by the advancing electrode, we verified the location of the electrode tip by ejecting coloured electrode fluid. Essentially four different images could be distinguished microscopically. (1) Injection into a cell. This led to sharply outlined, homogeneous and persistent staining of a single cell. (2) Injection into subcellular cystic spaces. This yielded a sharply defined, small, persistent coloured dot, which tended to grow slightly with time of ejection but never reached the size of a cell. Communication with the gallbladder lumen along the electrode shaft was frequently observed. (3) Injection into a LIS. Depending on the shape of the LIS this resulted in a more or less sharp outline of the space between two cells (Fig. 1), with occasional additional coloration of neighbouring spaces and faint coloration of the immediately underlying subepithelial space. In contrast to cell staining, the coloration of the LIS never persisted, disappearing within approximately 10 s of cessation of ejection from the electrode tip. (4) Injection into the subepithelial space. This resulted in diffuse coloration of a larger subepithelial area with a quite sharply defined front at the basal end of the epithelium but otherwise uncertain delineation. No backflux into the spaces

from underneath was observed. Depending on the amount of colour ejected, the subepithelial space cleared again in less than 1 min.

Although image (1) was correlated with the recording of a cell potential, the potential always dropped towards zero when the tip had entered a LIS (image (3)). In 10 measurements on 8 bladders a stable (> 1 min) potential difference of -1.61 (s.d. ± 0.96) mV (range -2.5 to $+0.8$) was recorded, LIS negative with respect to mucosal compartment. Referred to the serosal potential, which was measured immediately after each successful puncture by advancing the electrode further into the subepithelial space (image (4)), the potential of the LIS was 0.03 ± 0.83 mV negative which is not different from zero. (The trans-epithelial potential difference averaged 1.58 mV, lumen positive in these experiments.) We also continuously recorded the voltage divider ratio by passing square-wave constant current pulses of up to 100 μA , 1 s width and 0.1 Hz repetition rate across the bladder, and recording the potential deflections in the cell, the LIS and the subepithelial compartment. Expressing the data as fractional resistances (f) it was found that, on its way from the mucosal compartment into the LIS, the electrode tip had passed on average $97.8 \pm 4.7\%$ (range 90–104%) of the resistance between mucosal compartment and subepithelial space, indicating that the electrical resistance from LIS into the serosal fluid compartment was negligible. Image (2) was usually connected with a near-zero potential reading, but the simultaneously recorded fractional resistance was much lower than 100%.

In further experiments, we used double-barrelled micro-electrodes, with one barrel acting as K^+ -sensitive electrode and the other as reference. Because the localisation by fluid ejection was not always possible we determined the position of the electrode tip in some experiments from the potential and resistance profile and from the position of a lacquer mark which had been placed at a known distance of $\sim 40 \mu\text{m}$ from the tip. Thus, we assumed, in agreement with the above observations, that the tip had entered a LIS when, on advancing the electrode, the cell potential collapsed but f remained high, and when subsequent further advancement yielded a new cell potential (when the slightly diagonally moving electrode had crossed the LIS and penetrated the adjacent cell). The latter criterion as well as the depth of insertion, as read from the dye mark, distinguished LIS from subepithelial measurements.

Table 1 gives the results from eight stable measurements in six bladders. They yield a mean K^+ concentration for the LIS fluid of $3.9 \pm 0.8 \text{ mmol l}^{-1}$, which is significantly higher than the K^+ concentration of the mucosal and serosal bathing solution. To test the validity of our measuring technique we also determined the K^+ concentration in the subepithelial space by advancing the electrode from a LIS across the neighbouring cell into the underlying connective tissue. These measurements yield a mean K^+ concentration of $2.4 \pm 0.6 \text{ mmol l}^{-1}$ ($n = 9$) which is similar to that of the Ringer's solution. Although our measurements are not sufficiently advanced to yield complete concentration profiles along the LIS for all ions of interest (attempts to measure the Na^+ concentrations have been unsuccessful because of technical problems with the electrodes) we think that they open a new experimental approach towards a better understanding of the function of the LIS. Our observation that the K^+ concentration of the LIS is only slightly higher than that of the external bathing solutions calls for some caution with respect to the interpretation of concentration profiles from electron-probe data. Even though the latter data¹⁰, which show K^+ concentrations of up to 40 mmol l^{-1} , were obtained on a different tissue of a different species and at higher ambient K^+ concentrations (15 compared with 2.5 mmol l^{-1}), they are difficult to reconcile with the present findings, because there is no reason to believe that part of the space fluid K^+ should be bound (assuming that the sampling area of the electron beam does not include lateral folds² of the cell membranes).

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Deficient production of tyramine and octopamine in cases of depression

THE cause or causes of depressive illness are unknown, although biochemical evidence points to a physical basis for the psychiatric and somatic features of the disease¹. For nearly 20 years, the amine hypothesis² has been the most favoured explanation and putative derangements of noradrenaline and/or 5-hydroxytryptamine economy have been invoked as the systems of primary disturbance. Careful scrutiny, however, has revealed major inconsistencies in this hypothesis³. Some of the strongest evidence in its favour has been the ability of tricyclic antidepressant drugs to act as potent monoamine uptake inhibitors, thus potentially facilitating neurotransmission at the synaptic cleft; but recently, two drugs in this group, iprindole and mianserin, have been shown to be devoid of both noradrenaline and 5-hydroxytryptamine re-uptake-inhibiting ability, although each is a clinically effective antidepressant (for review see ref. 4). Two things should be pointed out in this connection. First, most pharmacological experiments performed to test the hypothesis have focused on noradrenaline and 5-hydroxytryptamine, whilst a possible role for the so-called trace amines⁵ has been almost completely neglected. And second, the direct clinical approach based on the detection of possible metabolic abnormalities has resulted in conflicting and controversial data³. We now report preliminary evidence pointing to a deficient production of two trace amines, tyramine and octopamine, in patients with depressive illness.

Thirteen females (mean age, 56.9 ± 3.2 yr) and 10 males (mean age, 57.6 ± 2.8 yr) suffering from primary depressive illness⁶ were studied. They had no history of mania or of significant physical illness. On admission, all were withdrawn from antidepressant or neuroleptic medication and placed on

placebo tablets for 7–10 days. In certain cases, night-time sedation (nitrazepam, 10 mg) was allowed for a few days. At the end of this 'drug-free' washout period, the patients were assessed for the severity of their disease by the Hamilton rating scale (HRS)⁷ for depression. The assessment on HRS was made by two independent raters who showed a satisfactory concordance ($r = 0.98$; $P < 0.001$). Only patients scoring 16 or more on the first 16 items of the HRS were included in the study. All were assessed on the Newcastle diagnosis scale⁸ and were assigned a diagnostic score placing them along an endogenous–non-endogenous dimension.

The control subjects were drawn from laboratory and hospital staff and were not on any form of medication. They included 17 females (mean age, 48.5 ± 2.2 yr) and 10 males (mean age, 52.9 ± 2.7 yr). Whilst no attempt was made to give patients or controls a standardised diet, patients received a full hospital diet during their drug-free period and controls living in hospital received a diet almost identical to that of the patients.

Mean 24-h urinary excretion values for free phenolic acids, total phenylacetic acid and free phenylethylamine in patients and controls are shown in Tables 1 and 2. Controls living in hospital had a similar excretion pattern of amine metabolites to those living outside hospital. The mean output of both *p*-hydroxyphenylacetic acid and *p*-hydroxymandelic acid (the major metabolites of *p*-tyramine and *p*-octopamine⁹) in depressed patients was significantly lower than that of controls (Table 1). When males and females were compared separately, the difference was also evident but only reached significance in females. There was no correlation with age. These results were examined in relationship to the patients' Newcastle scale scores. The three with high scores (9–11) on this scale, characterised by severe endogenous features and responding poorly to tricyclic drugs¹⁰, had very low excretion values of *p*-hydroxymandelic acid (4.64 ± 0.29 μmol per 24-h) and *p*-hydroxyphenylacetic acid (53.8 ± 13.0 μmol per 24-h) although the other metabolites were normal. The only other significant finding was that male depressives excreted more 4-hydroxy-3-methoxymandelic acid than females. These findings suggest that the deficit was a selective one and did not derive from some technical source of error such as incomplete sample collection.

The trace amines⁵ are represented in the brain in nanogram concentrations only. However, high tissue concentrations of catecholamines and 5-hydroxytryptamine stem not so much from increased production but from the existence of large storage depots. In addition, their assay is relatively simple whereas suitable quantitative procedures for the trace amines and their metabolites have become available only relatively recently⁵. It is important to note that the absence of a specific storage mechanism does not necessarily imply that turnover is any less rapid than that of compounds possessing one. Daily whole-body octopamine production, for example, falls little short of that of the individual catecholamines¹¹.

Although the urinary output values of trace amine breakdown products which we have measured in depressive illness for the

Table 1 Urinary excretion of total phenylacetic acid (PAA) and free hydroxyphenylacetic (HPAA), homovanillic (HVA), *p*-hydroxymandelic (*p*-HMA) and 4-hydroxy-3-methoxymandelic (VMA) acids in control subjects and depressive patients

Group	n	PAA	o-HPAA	Urinary excretion (μmol per 24 h)				
				m-HPAA	p-HPAA	HVA	p-HMA	VMA
Depressive patients								
Males	10	911 ± 66	5.85 ± 0.85	27.2 ± 6.1	80.1 ± 14.7	21.6 ± 2.6	8.74 ± 1.84	27.1 ± 2.9†
Females	13	808 ± 140	4.93 ± 0.92	30.9 ± 5.8	90.9 ± 9.7†	18.0 ± 1.0	5.71 ± 0.89‡	18.6 ± 1.8‡
All patients	23	852 ± 95	5.32 ± 0.66	29.3 ± 4.1	86.2 ± 8.3*	19.5 ± 1.3	7.02 ± 1.01§	22.2 ± 1.8
Controls								
Males	10	896 ± 132	8.41 ± 1.38	34.6 ± 8.4	128.9 ± 23.6	25.2 ± 2.2	12.07 ± 1.90	23.0 ± 2.0
Females	17	881 ± 154	5.45 ± 0.46	40.5 ± 6.2	146.7 ± 15.8†	20.6 ± 1.4	9.46 ± 1.31‡	23.5 ± 1.6
All controls	27	881 ± 110	6.57 ± 0.66	38.3 ± 4.9	140.1 ± 13.1*	22.3 ± 1.0	10.41 ± 1.07§	23.3 ± 1.2

Urine samples (24-h) from each group were collected into 25 ml 6 M HCl and stored at –15°C. Free phenolic acids (*o*-, *m*-, and *p*-hydroxyphenylacetic acids, homovanillic acid, *p*-hydroxymandelic acid and 4-hydroxy-3-methoxymandelic acid¹⁶ and total phenylacetic acid¹⁷ were measured by capillary column flame-ionisation gas chromatography. Phenylacetic acid, *p*-hydroxyphenylacetic acid and *p*-hydroxymandelic acid are the major metabolites, respectively, of phenylethylamine, *p*-tyramine and *p*-octopamine which are, themselves, synthesised sequentially from L-phenylalanine⁹. Values are given as means ± s.e.m.

* All controls versus all depressives $P < 0.005$. † Female controls versus female depressives $P < 0.01$. ‡ Male depressives versus female depressives $P < 0.02$. § All controls versus all depressives $P < 0.05$. ‡ Female controls versus female depressives $P < 0.05$.

Table 2 Urinary excretion of free phenylethylamine in control subjects and depressive patients

	<i>n</i>	Urinary excretion of free phenylethylamine ($\mu\text{mol per 24 h}$)
Control subjects		
Males	10	38.7 $\pm$ 5.78
Females	16	40.9 $\pm$ 6.77
Total	26	40.0 $\pm$ 4.70
Depressed patients		
Males	10	51.6 $\pm$ 13.1
Females	12	39.2 $\pm$ 7.76
Total	22	44.8 $\pm$ 8.25

Free phenylethylamine was determined by an electron-capture gas chromatographic method¹⁸. Values are means $\pm$ s.e.m.

first time appear to support this view, it must be remembered that an unknown proportion of these acidic metabolites may derive from diet or gut flora (for extended discussion, see ref. 9). *p*-Hydroxyphenylacetic acid and *p*-hydroxymandelic acid, which presumably reflect quantitatively the turnover of their precursor amines, are metabolically interconnected (Table 1). Even though changes in phenylethylamine production are likely to have been masked by a massive gut flora contribution⁹, our recent findings of significantly low concentrations of phenylacetic acid, the major metabolite of this amine, in cerebrospinal fluid from patients with depressive illness¹² suggests that phenylethylamine too may be deficient in the central nervous system of these patients.

Note also that an as yet unknown proportion of the total production of both tyramine and octopamine derives from the periphery, particularly the sympathetic nervous system and the tissues it innervates¹³. However, even if the changes we have described were to prove to be wholly peripheral, and such a finding need not necessarily detract from their relevance to depression, this disease may well turn out to stem from a generalised upset, connected perhaps with a membrane transport deficit, rather than a disturbance localised solely to the brain¹⁴.

What lessons are we to draw from the present findings? We have no wish to substitute a tyramine-octopamine hypothesis for its competitors. Recent data implicating histamine H₂ receptors in the therapeutic response to tricyclic antidepressant drugs⁴ are particularly compelling but by no means exclude other factors. It seems that an extremely complex sequence of events is responsible for the maintenance of normal affect, in which histamine, catecholamines, 5-hydroxytryptamine, and the trace amines also, may all play their part. Recent trials have indicated that when administered orally, phenylalanine, the precursor of phenylethylamine, tyramine and octopamine, may be beneficial in certain patients with depressive illness¹⁵.

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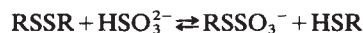
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Sulphonation of cholinergic receptor disulphide bond increases response to ACh

THE presence on the cholinergic receptor of a critical disulphide bond has been demonstrated and localised to within 10 Å of the cholinergic receptor anionic binding subsite¹⁻⁴. Reduction of this bond by dithiothreitol results in a reduced response of the nicotinic cholinergic receptor to acetylcholine (ACh) in many systems^{1,5-8}. I report here that heterolytic cleavage of this disulphide bond is produced using sodium bisulphite, a reagent which shows a high degree of specificity, reactivity and reversibility of interaction with the disulphide bond⁹. The active agent is the bisulphite ion, the reaction being



and resulting in the addition of the strongly nucleophilic sulphonate group to the receptor. The addition of this group seems to modify the ACh receptor-ionophore complex in a unique manner which results in an increase in its response to ACh.

The preparation used was the cutaneous pectoris muscle of the frog *Rana pipiens*. Reagents were added to continuously perfusing frog Ringer (NaCl 115 mM, KCl 2.0 mM, CaCl₂ 0.2 mM, MgCl₂ 5.0 mM, buffered with HEPES, pH 7.2, at room temperature). Sodium bisulphite was effective in concentrations of 10⁻⁵-10⁻³ M. All compounds were bath applied with the exception of the ACh iontophoretic experiments. The amplitude of either the miniature end plate potential (m.e.p.p.) or iontophoretically-induced ACh responses were used as a measure of the postsynaptic response to ACh.

The application of 1 mM NaHSO₃ to the preparation results in an increase in m.e.p.p. amplitude within 5 min, most typically with a 1-min latency. This increase may be up to 300%, although increases of 50-100% are more common. The amplitude increase is not the result of a single class of large m.e.p.ps but rather a shifting of the entire amplitude spectrum to the right (Fig. 1a, b). Iontophoretic application of ACh shows this amplitude increase to be of postsynaptic origin (Fig. 1c). The rise or delay time of the response to ACh (m.e.p.p. or iontophoresis) and the input impedance of the postsynaptic membrane (measured with two intracellular electrodes) are unchanged by bisulphite. The resting potential of the postsynaptic membrane remains constant even after a 2-h application of bisulphite in concentrations up to 5 $\times$ 10⁻³ M. (Presynaptic effects of bisulphite, including m.e.p.p. frequency increases shown in Fig. 1a, will be reported elsewhere.)

From the above data, it can be concluded that the increased response to ACh following bisulphite application cannot be attributed to changes in the postsynaptic membrane input resistance (no change in input impedance), to an action on acetylcholinesterase (no change in response decay time) or to changes in presynaptic release (response to iontophoretic ACh increases). An action on the postsynaptic cholinergic receptor molecule thus seems a possibility. As the bisulphite ion has a high degree of specificity of reaction with disulphide bonds⁹ and as a disulphide bond on the cholinergic receptor has been shown to be critical in postsynaptic ACh action¹⁻⁴, the following experiments were carried out using compounds known to

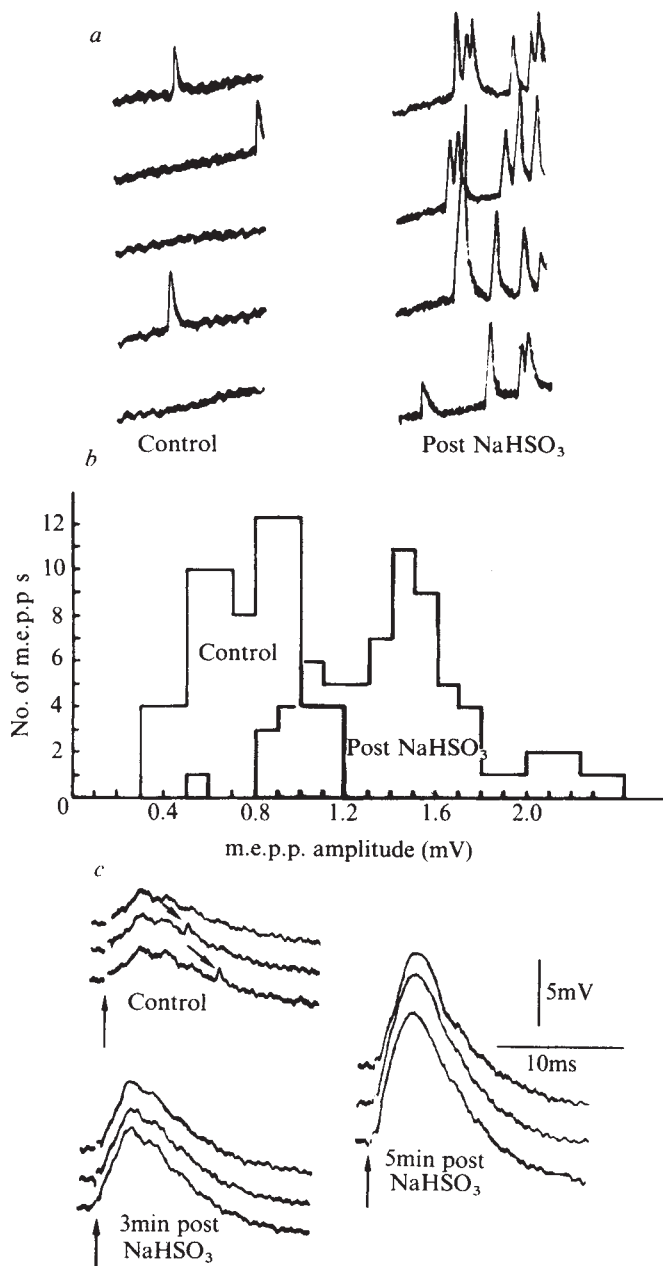


Fig. 1 Increases in response to ACh following sodium bisulphite. *a*, Intracellularly recorded m.e.p.s. before and 15 min after NaHSO₃ application (10^{-3} M); *b*, m.e.p.p. amplitude spectrum of the same muscle fibre during control recording and 15 min after NaHSO₃ treatment (10^{-3} M); *c*, response of postsynaptic membrane to iontophoresed ACh (large arrows) before and after NaHSO₃ application (10^{-3} M). Small arrows indicate m.e.p.s.

localise the site of action of a reagent to this disulphide bond of the cholinergic receptor^{1-4,8}.

DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] forms an addition product with sulphhydryl groups which then revert to the oxidised state of the disulphide bond⁹. It will reverse the reduction of the disulphide bond on the cholinergic receptor produced by dithiothreitol¹. The effect of DTNB on the bisulphite-induced amplitude increase was thus tested. Application of DTNB (0.2×10^{-3} M) after bisulphite treatment (10^{-3} M) produces a rapid return of the m.e.p.p. amplitude to control values (Fig. 2). DTNB, without prior bisulphite treatment, has no effect on the end plate at this concentration. The accelerated reversal of the bisulphite response by DTNB most probably occurs by combination of DTNB with free -SH groups and -SH groups produced by hydrolysis of the easily reversible sulphonate⁹.

MBTA [4(*N*-maleimido)- α -benzyltrimethylammonium-iodide] was synthesised as an affinity label for sulphhydryl groups located near the cholinergic binding site². MBTA alkylates covalently at its maleimide group and binds reversibly at the quaternary end of the molecule to the cholinergic ligand binding site. Through the irreversible alkylation, MBTA prevents restoration of the disulphide bond by DTNB and at the same time keeps its quaternary group in the vicinity of the cholinergic binding site where it competes with ACh for the receptor site². Application of MBTA (5×10^{-7} M), following application of bisulphite, reduces the postsynaptic response to ACh, presumably by competitive inhibition of the quaternary group of MBTA with the spontaneously released ACh. It also prevents the DTNB reversal of the bisulphite response to control values (Fig. 3). MBTA without prior bisulphite treatment has no effect either pre- or postsynaptically at this concentration.

Occupation of the receptor binding site by ACh has been shown to prevent the reaction of dithiothreitol with the receptor disulphide bond⁸. The experiment was repeated using sodium bisulphite and ACh to ascertain whether the binding of ACh to the receptor would also prevent the action of the bisulphite ion on the receptor. Following a control m.e.p. recording, the muscle was exposed for 2 min to ACh (5×10^{-6} M) and then for 20–40 min to ACh (5×10^{-6} M) plus sodium bisulphite (3×10^{-3} M). This concentration of bisulphite would invariably have produced a high m.e.p.p. frequency and large amplitude increase. However, during application and the subsequent 40 min Ringer wash, no change in m.e.p.p. amplitude and only a small change in m.e.p.p. frequency occurred. The contralateral muscle used as a control showed the usual bisulphite effect. These experiments were also repeated with fibre sampling: 20 fibres were sampled, at each experimental point above, with the same results. Occupation of the receptor by ACh seems to inhibit disulphide bond modification either by preventing access to the bond by steric hindrance or by induction of a conformational change after which the disulphide bond is either inaccessible or no longer essential for further receptor-ionophore interaction. Evidence that steric hindrance is not the mechanism of ACh protection is given by the fact that curare, a bulky molecule which binds to the cholinergic receptor, is

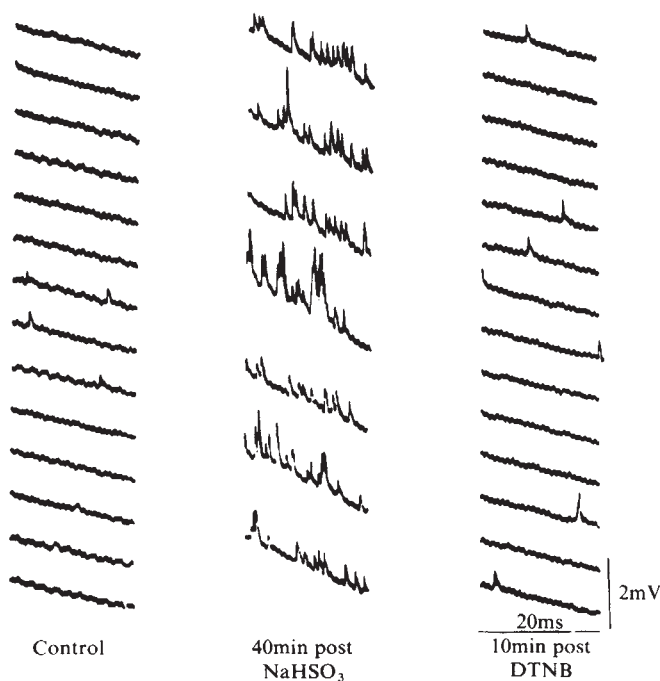


Fig. 2 DTNB reversal of m.e.p.p. amplitude and frequency increase following NaHSO₃ application (10^{-3} M). DTNB (0.2×10^{-3} M) was applied 40 min after NaHSO₃ application, when m.e.p.p. frequency and amplitude were maximal. Reversal begins approximately 2 min post-DTNB and is complete at 20–40 min.

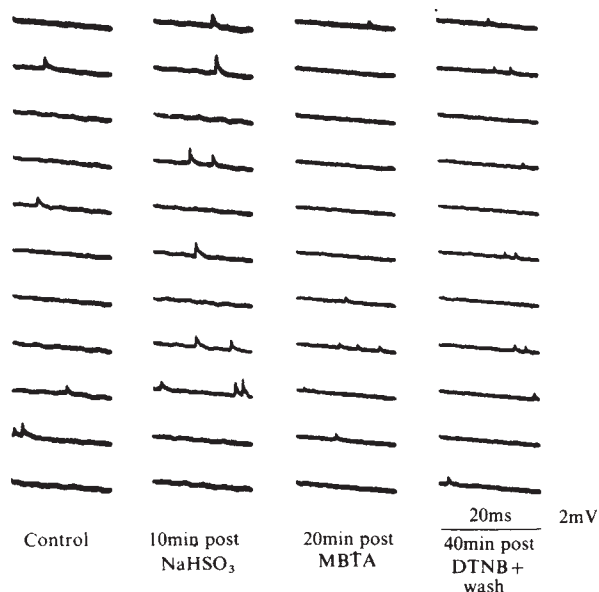


Fig. 3 Use of alkylating affinity label MBTA to demonstrate the presence of the sulphhydryl group produced by NaHSO_3 application. MBTA (5×10^{-7} M) is applied after bisulphite has produced a m.e.p.p. amplitude increase. The production of a sulphhydryl group by bisulphite is shown by the presence of affinity alkylation as demonstrated by (1) failure of DTNB to produce reversal of the MBTA response and (2) reduced m.e.p.p. amplitude produced by competition of spontaneously released ACh with the quaternary group of the affinity label.

unable to block the action of dithiothreitol⁸. Desensitisation of the ACh receptor does not seem to be involved in the process of ACh protection, as it has been shown that receptors in the desensitised state are still accessible for reduction by dithiothreitol⁸.

The above data demonstrate an action of bisulphite on the postsynaptic cholinergic receptor which increases the receptor response to ACh. Bisulphite is known to produce a heterolytic cleavage of disulphide bonds with resultant addition of a sulphonate group⁹. This action has been localised to a disulphide bond in the vicinity of the receptor negative subsite by the use of MBTA and is likely to be the same disulphide bond which has been reduced by dithiothreitol in previous experiments¹⁻⁸. The mechanism of action of this increased response to ACh following sulphonation remains unclear, but must be linked to the addition to the receptor molecule of the negative charge of the sulphonate group.

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A semi-synthetic penicillinase inactivator

MANY pathogenic bacteria secrete penicillinases (β -lactamases, EC 3.5.2.6) as a defence mechanism against β -lactam antibiotics¹. Inhibitors of penicillinase are therefore of potential clinical importance, and interest in this area has been stimulated by the discovery of effective naturally occurring enzyme inactivators²⁻⁴. We have studied the interaction of one of these, clavulanic acid (I) with the exocellular enzyme from *Staphylococcus aureus*, and we report here the synthesis of a novel penicillinase inhibitor whose design was based on the mechanism postulated for the inactivation by clavulanate.

Sodium clavulanate rapidly inactivates the penicillinase from *S. aureus* with close to 1:1 stoichiometry. Partially inactivated enzyme regains 10-15% activity over 10 min at pH 7 and 25 °C; fully inactive enzyme can be prepared by treatment with a small excess of inactivator. The apparent K_m for inactivation is 12 μM , and the enzyme is protected from inactivation by good substrates such as the chromogenic cephalosporin, 87/312 (ref. 5), or by competitive inhibitors such as phenylacetylcephalosporin C. Clavulanate is therefore a highly specific active site-directed inhibitor of *S. aureus* penicillinase.

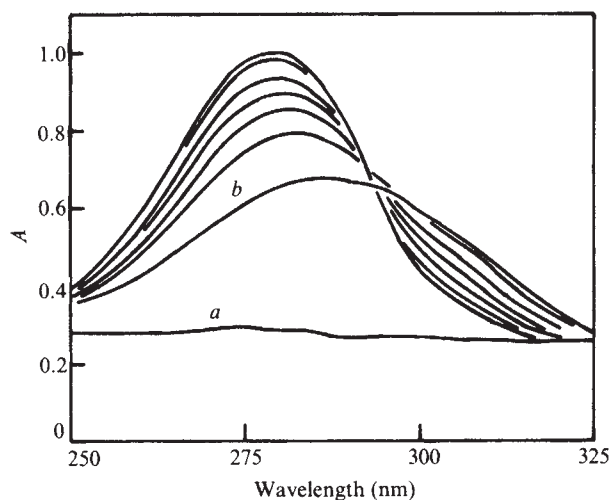
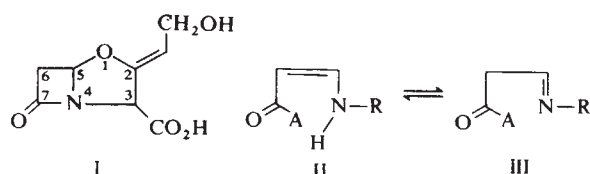


Fig. 1 Difference spectra, (enzyme + clavulanate) - enzyme, for an equimolar mixture of enzyme and inhibitor, 3.5×10^{-5} M, at pH 8 and 25 °C. a, Is the baseline, b, the spectrum immediately after mixing. The successive spectra with isobestic point 292 nm were taken 1, 3, 6, 10, 20 and 35 min after mixing.

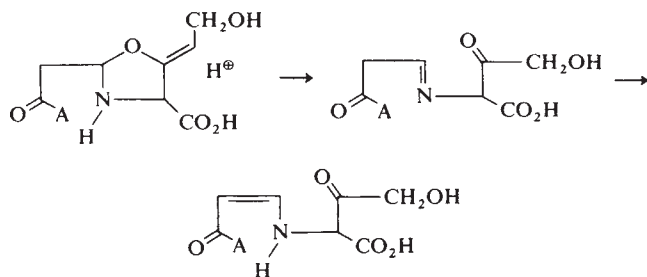
Incubation of the protein with clavulanate leads to the appearance of a new component in the UV spectrum, whose evolution is shown in Fig. 1. Loss of activity is associated with the rapid appearance of a species with maximum absorbance at wavelength (λ) 290 nm. In a slower process, not accompanied by any change in the extent of inactivation, this species is converted into another, with $\lambda_{\text{max}} = 278$ nm and extinction coefficient $21,500 \text{ cm}^{-1} \text{ M}^{-1}$. Inhibited enzyme can be fully reactivated by incubation in acid solution. Figure 2 shows that this restoration of activity is paralleled by loss of absorbance due to the UV chromophore. The kinetics of the reactivation were measured between pH 5 and 10.5. In this range the rapid phase of the reaction is first order, with the rate constant decreasing from 0.12 min^{-1} at pH 5 to $<0.001 \text{ min}^{-1}$ at pH 10.5. The slow phase leading to complete reactivation shows a similar pH dependence. Hydroxylamine has no effect on the reaction.

The simplest hypothesis to explain these results is that inhibition is due to formation of a compound of partial structure II from the β -lactam ring of clavulanate. 'A' represents an enzyme



active site residue. We suggest that this structure is an analogue of an acyl enzyme in the normal reaction pathway, stabilised to hydrolysis by the unsaturation at C5–C6 (ref. 6). A compound containing this structure (ethyl β -benzylaminocrotonate) has $\lambda_{\max}$ 286.5 and 275.5 nm, and extinction coefficients 23,400 and 28,800 M⁻¹ cm⁻¹ in the *cis* and *trans* forms, respectively (in 95% ethanol)⁷, and these values are typical of β -amino- α , β -unsaturated carbonyl compounds⁸. We suggest therefore that the slow change in spectrum (Fig. 2) is due to *cis-trans* isomerisation. Structures of type II are expected to be unstable in acid because of tautomeric equilibria with imines (III), whose hydrolysis is acid catalysed. A model compound, ethyl 3-carboxymethylamino-2-butenate, synthesised by condensation of ethylacetoacetate and glycine, showed the expected spectrum and acid instability.

If this proposal for the mechanism of inactivation is correct, the difference that makes clavulanate an inactivator of the enzyme and closely similar compounds, such as the penicillins (IV) substrates, is the possibility of an elimination across the C6–C5 bond. This elimination could occur by a two-step route



or, as proposed by Charnas *et al.*⁹, by single-step abstraction of a proton from C6 by an enzymatic base. It is not possible to decide between these routes on the basis of present evidence. Either way, elimination requires a sufficiently good leaving group at C5, and a proton which can be lost from C6.

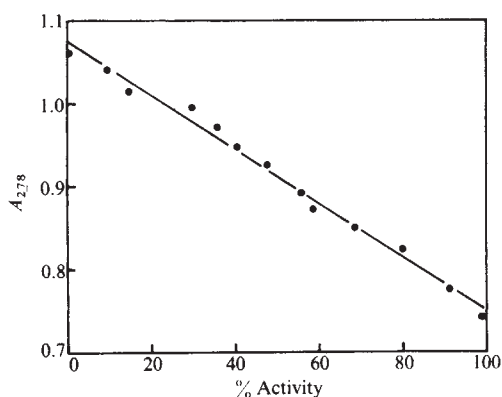
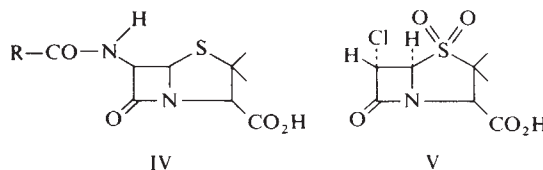


Fig. 2 Correlation of restoration of activity with loss of UV absorbance. Penicillinase (2.7×10^{-5} M) in 0.1 M sodium acetate buffer, pH 5.0, was incubated at room temperature with an equal concentration of clavulanate. The absorbance at 278 nm and the enzymatic activity were measured at intervals over the next 3.5 h.

Oxygenation of sulphur makes it a better leaving group¹⁰, and we therefore synthesised penicillin G sulphone¹¹. This compound was found to be a good substrate, with K_m 20 μ M and $V_{\max}$ the same as for penicillin G. The sulphone does not inactivate the enzyme, but a transiently formed (non-enzyme-containing) hydrolysis product has $\lambda_{\max}$ 280 nm. Presumably, in this case the elimination only occurs after the penicilloic acid has left the active site.

Penicillins lack one of the two hydrogen atoms at C6 in clavulanate and elimination by the single-step route may be sterically impossible in penicillins and derivatives. Alternatively, the hydrogen atom in penicillin G sulphone may be too weakly acidic. 6-Chloropenicillanic acid, as usually synthesised¹² (but see also ref. 13), has the opposite configuration at C6 to that in penicillins, and such an electronegative substituent presumably has an acid-strengthening effect on the hydrogen atom. We therefore synthesised 6- α -chloropenicillanic acid, and found it to be a poor substrate for the enzyme (K_m = 3–5 mM, $V_{\max}$ 0.8 relative to penicillin G = 100). The compound is not an inactivator of the enzyme. Oxidation of 6- α -chloropenicillanic acid with $KMnO_4$ gave the novel 6- α -chloropenicillanic acid sulphone (V).



Incubation of the enzyme with this compound leads to inactivation similar to that produced by clavulanate, except that (1) no reactivation of the enzyme is observed at pH 7, and (2) the enzyme hydrolyses about 100 molecules of the compound for each molecule of the enzyme that is inactivated. This behaviour is thus similar to that found for the reaction of clavulanate with the *Escherichia coli* enzyme^{9,14}. The apparent K_m for the inactivation process is about 100 μ M. We anticipate that the stability of the inactivated form of the enzyme will allow identification of the site of attachment of the inhibitor.

English *et al.* have reported¹⁵ that unsubstituted penicillanic acid sulphone inhibits penicillinases, including that from a strain of *S. aureus*. It seems likely that the mechanism of this inhibition is similar to that proposed here.

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In vivo proteolytic cleavage of colicins requires specific receptor binding

COLICINS are plasmid-specified antibiotic proteins produced by some strains of coliform bacteria¹. They are distinguished from other bacterial antibiotics such as aminoglycosides and tetracyclines by their large molecular weights (40–80,000) and their narrow host range. Colicins only kill bacteria having the appropriate cell-surface receptors^{1,2}, and even then the ability of a cell to produce a given colicin usually confers immunity to killing by that colicin. We are interested in the comparative properties of colicins and their similarities to bacterial toxins that are active against mammalian cells, some of which are also encoded by extra-chromosomal elements. A fundamental question concerning the action of both colicins and toxins is how these large, essentially hydrophilic proteins traverse the mainly hydrophobic cell surface to reach the lesion sites. For colicins these include DNA for colicin E2 (ref. 3), RNA (colicin E3 and cloacin DF13)^{4,5}, and presumably the inner cell membrane, where membrane potential and energy metabolism are affected (colicins E1, K and Ia (refs 2, 6, 7)). Here, we show that binding of colicin to its specific receptor can result in a specific proteolytic cleavage to generate two peptide fragments. The smaller of these, derived from the C-terminal region of the colicin, has a greater specific biological killing activity *in vitro* than the parent molecule. We propose that *in vivo* the proteolytically derived

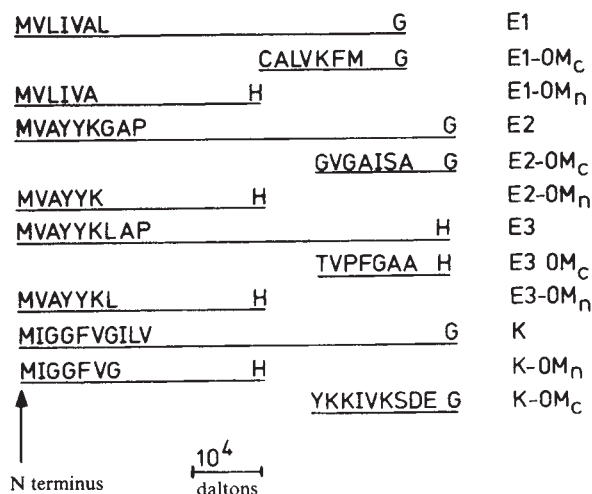


Fig. 1 For analytical purposes, 0.17 nmol purified colicins¹⁰ were incubated with 0.1 A_{600} units of *Escherichia coli* K12 outer membranes¹² in 0.1 M sodium phosphate buffer (pH 7.0) for 15 min at 37 °C. Membranes were removed by centrifugation (78,000g, 90 min) and the peptides resolved by Laemmli SDS (0.1%) polyacrylamide slab gel electrophoresis¹³ (4.5% stacking gel, 12.5% running gel). Co-run MW markers were used in the determination of the MWs of the colicin polypeptides. For preparative gels, 13.3 nmol of digested colicin was run in wide tracks on each gel. Each colicin sample was flanked by a track containing MW markers. The latter tracks only were stained and by reference to the analytical results, each band containing a presumptive colicin-derived polypeptide was cut out, disrupted in distilled water (18 h, 4 °C) and finally dialysed extensively against distilled water. 90% of the product (about 50 nmol) was lyophilised, and then used in determination of N- and C-terminal residues and N-terminal sequences^{17–19}. The remaining sample was precipitated by $(\text{NH}_4)_2\text{SO}_4$ (70% w/v) and dialysed against the appropriate reaction buffer before assaying for *in vitro* activities. Intact colicins were treated in the same way before structural determination and *in vitro* assay. C-terminal peptides = OM_c; N-terminal peptides = OM_n. For example, E1-OM_c = C-terminal colicin E1 polypeptide derived from incubation of colicin E1 with outer membranes.

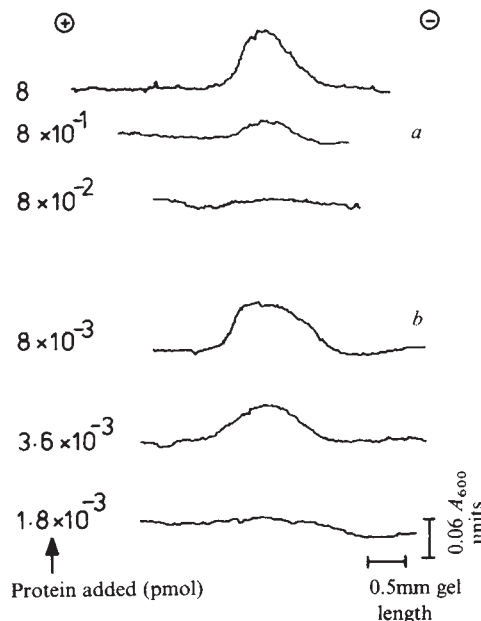


Fig. 2 Colicin E3(a) or E3-OM_c(b) were incubated with ribosomes from sensitive K12 cells²⁰ using the polyacrylamide gel assay method of Lau and Richards⁴. The amount of the nucleotide fragment generated from 16S rRNA was quantified using a Joyce-Loebel densitometer. 80–500 pmol E1-OM_c, K-OM_c and E2-OM_c, colicins E1, E2 and K and all OM_n fragments (Fig. 1) gave no measurable generation of nucleotide fragment (similar traces to the bottom one in a).

C-terminal fragment is transported across the cell surface to the lesion site. This process seems analogous to the apparent cleavage of diphtheria toxin during its entry into mammalian cells⁸.

Because colicins E2 and E3, and cloacin DF13 can be cleaved by pure proteases to yield specific polypeptide fragments, some of which have the nuclease activity of their parent colicin *in vitro* but not *in vivo*^{4,9–11} it seemed possible that colicins, after binding to their specific receptors on whole cells, might be proteolytically cleaved to generate a biologically active fragment which is then transported to the lesion site. To test this for colicins E2, E3, E1 and K, we incubated purified colicins separately with outer membrane vesicles¹², derived either from colicin-sensitive cells (receptor intact) or from resistant cells (receptor non-functional). Incubation of each colicin with colicin-sensitive outer membrane vesicles resulted in a complete conversion of each colicin to two peptides, derived from each end of the colicin molecule (Fig. 1). In contrast, vesicles from an E-colicin-resistant, K-colicin-sensitive (E^+K^+) strain cleaved colicin K but not E, whereas vesicles from an E^+K^- strain cleaved E but not K colicins. None of the colicins were cleaved by outer or inner membranes from E^-K^+ strains or by inner membrane vesicles from sensitive cells. Lysozyme, ovalbumin, cytochrome *c* and bovine serum albumin were not cleaved during incubation with any of the vesicles.

Although none of the fragments isolated from preparative SDS-polyacrylamide gels¹³ killed whole cells, even after extensive dialysis, the C-terminal peptides derived from colicins E2 and E3 showed activity in *in vitro* assays. Moreover, their specific activities were higher than those of their parent colicins isolated in the same way. The specific activity of the colicin E3 C-terminal fragment was 300–1,000 times greater than that of the intact colicin (Fig. 2). Colicin E2 DNase specific activity was about 1% of that of its C-terminal peptide (Fig. 3).

In an attempt to assay the *in vitro* activities of colicins E1 and K and their polypeptide fragments, we used the enhanced fluorescence assay of Phillips and Cramer¹⁴. Incubation of colicin E1 (Fig. 4a) or colicin K (Fig. 4b) with intact sensitive cells in

the presence of 8-anilino-1-naphthalene sulphonic acid (ANS) resulted in a large rapid increase in fluorescence, whereas incubation with colicin-resistant cells showed no fluorescence increase. The much smaller increase in fluorescence observed when intact colicins E1 and K were incubated with outer membranes of $E^s K^s$ cells could reflect the binding of colicins to their receptors on the outer membrane. Incubation of C-terminal colicin E1 (E1-OM_c) and C-terminal colicin K (K-OM_c) peptides with inner membranes resulted in a slow but reproducible increase in fluorescence. This may reflect a significant interaction of these polypeptides with their presumptive lesion site in the inner membrane, as N-terminal colicin E1 and K peptides (E1-OM_n, K-OM_n), and colicins E2 and E3 and their peptides had no such effect. If these interactions with inner membranes are of significance, then E1-OM_c and K-OM_c are more active than their respective whole colicins.

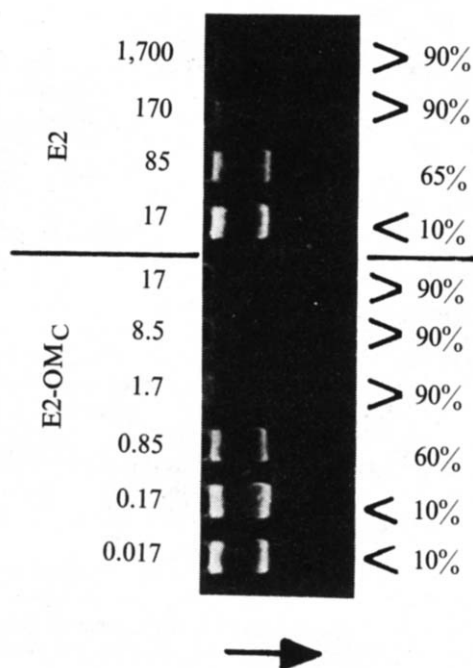


Fig. 3 DNase activity of colicin E2 and its polypeptide fragments. Colicins or their peptides (Fig. 1) were incubated with 0.6 μ g bacteriophage λ DNA for 1 h at 37°C in 16 μ l of the reaction mixture of Schaller and Nomura³. Samples were then assayed on a 0.9% agarose gel³. The direction of DNA migration is indicated. Much of the undigested λ has remained at the origin of the gel. We have noted this with many DNA preparations, and it could result from the binding of DNA to non-DNA macromolecules present as contaminants in the DNA preparation. The amount of protein ($\text{mol} \times 10^{-15}$) added for each assay is indicated above each track, and the degree of digestion is indicated below. Colicins E1, K, E3, their peptides and E2-OM_n (not shown), and bacteriophage λ incubated in the absence of added protein, showed no detectable digestion.

Although *in vitro* target activity is associated with the C-terminal fragments, all N-terminal peptides bound cell-surface receptors (Fig. 5). Furthermore, additions of N-terminal fragment in a 10^4 – 10^5 -fold molar excess to homologous colicin inhibited killing activity. Predictably, K-OM_n did not interfere with E-colicin killing and N-terminal colicin E fragments did not affect K-colicin killing. None of the C-terminal fragments effected *in vivo* killing by any colicin.

In conclusion, these data demonstrate that after cell-receptor binding, colicins can be proteolytically cleaved into two peptides with mutually exclusive receptor binding and killing activities. This seems analogous to the proposed action of diphtheria toxin on mammalian cells⁸. Common factors include protein fragmentation into large (molecular weights 38,000 and 35–36,000)

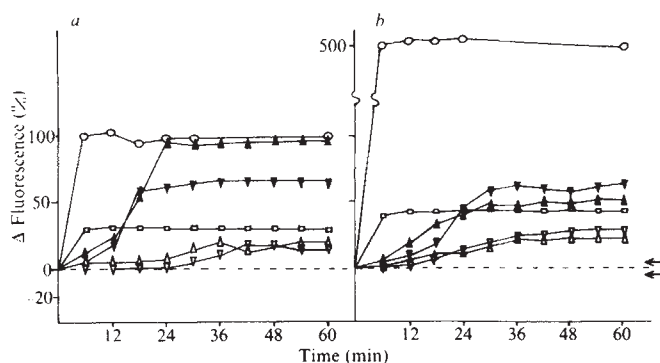


Fig. 4 Fluorescence changes (502 nm) after colicins and their polypeptide fragments (71 pmol per assay; Fig. 1) were added (0 min) to 8 A_{600} units of the indicated cell preparation in 1 ml of M9 salts, equilibrated with ANS (60 μ M). *a*, Colicin E1-derived polypeptides; *b*, colicin K-derived polypeptides. $\circ$, Intact colicin + $E^s K^s$ cells; Δ , intact colicin + inverted spheroplasts²¹ of $E^s K^s$ cells; ∇ , intact colicin + inner membranes¹² of $E^s K^s$ cells; $\square$, intact colicin + outer membranes¹² of $E^s K^s$ cells; $\blacktriangle$, -OM_c peptides + inverted spheroplasts of $E^s K^s$ cells; $\blacktriangledown$, -OM_c peptides + inner membranes of $E^s K^s$ cells. The following combinations of colicin-derived peptides (71 pmol) and cell preparations gave <2% fluorescence change at any time during the 60-min incubation (values within the parallel arrows). (1) Any cell preparation incubated with E1-OM_n; K-OM_n; colicin E2, E3 or any of their fragments. (2) Whole $E^s K^s$ cells or their outer membranes incubated with E1-OM_c and K-OM_c. (3) $E^s K^s$ cells and their outer membranes incubated with intact colicins.

cell binding and small (24,000 and 19–21,000) *in vitro* killing fragments, neither fragment having *in vivo* activity. However, although the action of diphtheria toxin requires cleavage of disulphide bonds, none are present in E and K colicins (D.H.W., unpublished). Another common feature between colicins, diphtheria toxin and some other bacterial toxins is that they are encoded by extra-chromosomal elements: this may have facilitated their spread among bacteria and their evolution¹⁵. Specific cell-surface-associated proteolysis is clearly a key step in the action of some bacterial toxins on bacterial and mammalian cells: whether this same protease is responsible for the cleavage and release of secreted proteins¹⁶ remains to be seen.

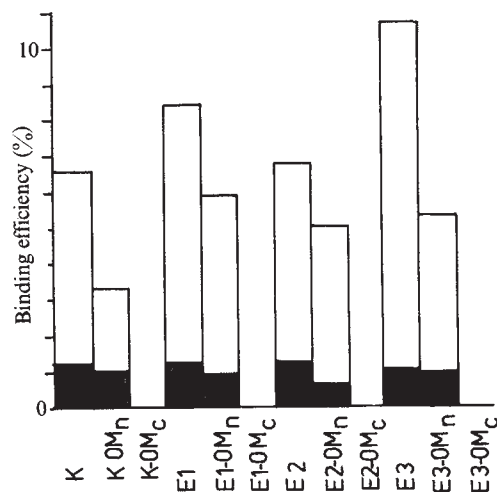


Fig. 5 Five nmol ^{14}C -labelled colicin or proteolytic fragments^{22,23} (see Fig. 1: 65.3 – 161.2×10^3 c.p.m. per 5 nmol) were incubated with sensitive (upper bar) or $E^s K^s$ cells (lower bar, shaded) (10^8 cells ml^{-1}) for 30 min in 1 ml L-broth. After centrifugation and resuspension (boiling 1% SDS), the cell pellet was counted on 1 MM Whatman paper using 0.15% diphenyloxazole in toluene.

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Induction of erythroid differentiation in the human leukaemia cell line K562

MURINE erythroleukaemic cell lines can be induced by various low molecular weight compounds to differentiate *in vitro*¹. Corresponding human cell lines have not previously been described but we now report that human leukaemic cells of the K562 cell line can be induced to red cell differentiation *in vitro*.

Table 1 Accumulation of erythrocyte-like, benzidine-positive particles in sodium butyrate treated cultures

Days in culture	Per cent of benzidine positive particles*				
	0	1	2	3	4
1 mM Na-butyrate	1.4	3.9	12	35	48
Control	1.4	0.9	0.9	1.1	1.4

*Cytocentrifuged smears were stained with benzidine-Giemsa. Percentages were calculated by counting more than 300 cells.

The K562 line was originally established by Lozzio and Lozzio from the pleural effusion of a patient with chronic myelogenous leukaemia in terminal blast crisis². This cell line, which has been considered to represent an early differentiation stage of the granulocyte lineage³, is commonly used as a sensitive target cell in the human natural killer cell assay⁴.

We have recently analysed the surface glycoprotein patterns of various established human haematopoietic cell lines^{5,6} and those of isolated populations of normal and leukaemic leukocytes⁷⁻⁹. The surface glycoprotein pattern of K562 cells was found to be clearly different from those of benign and malignant cells representing various stages of the myeloblast to granulocyte differentiation sequence. In contrast, we observed several common features of normal erythrocytes and K562 cells. These observations led us to investigate more closely the K562 cells.

The cells were cultivated in RPMI 1640 culture medium supplemented with 10% newborn-calf serum. K562 cells carry on their surface¹⁰ and synthesise¹¹ glycophorin A, which is the major sialoglycoprotein on human red cells¹². This provides additional evidence for the erythroid nature of the K562 cells, as glycophorin A is known to be expressed exclusively on basophilic normoblasts and on later stages of the red cell differentiation in human bone marrow¹³. The K562 cells evidently also contain spectrin as seen by indirect immunofluorescence using rabbit anti-spectrin antibodies on acetone-fixed smears (data not shown).

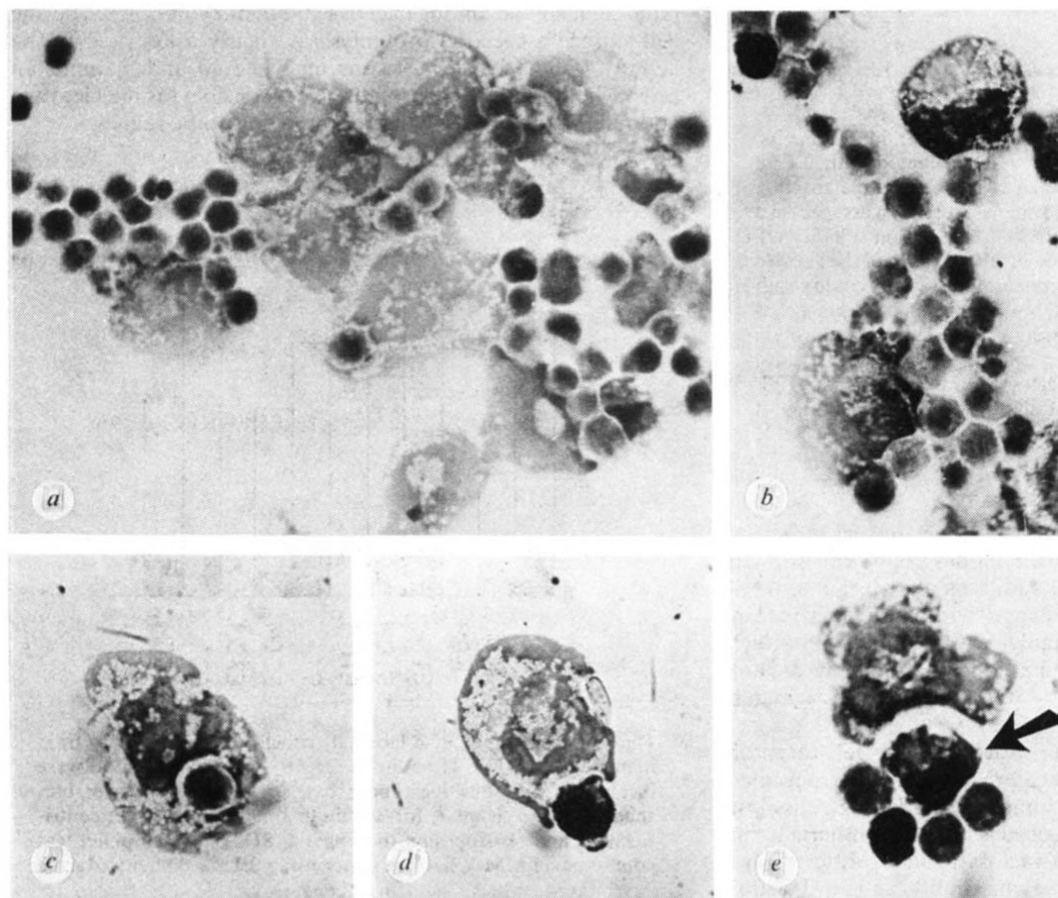


Fig. 1 Microphotographs of cytocentrifuged smears of K562 cells cultivated for 4 days in the presence of 1 mM sodium butyrate. *a*, Stained with Giemsa-eosin and photographed using a green filter. *b*, Stained with Lephene's benzidine method-Giemsa and photographed using a red filter. *c-e*, Details of the apparent sequence of generation of erythrocyte-like particles in butyrate induced K562 cultures. The arrow indicates a normoblast-like cell. These were stained with Giemsa-eosin and photographed with a green filter.

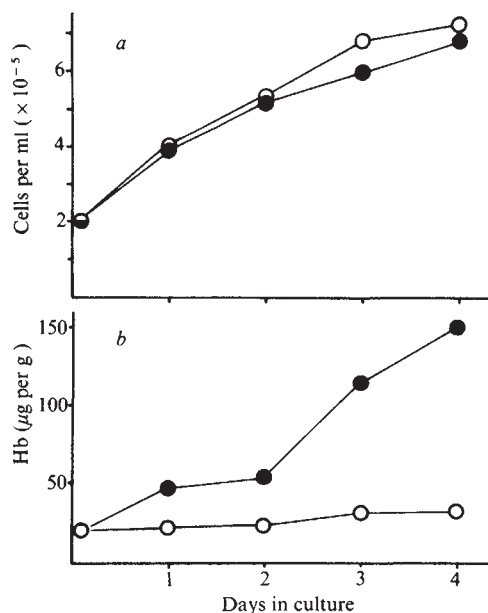


Fig. 2 *a*, Cell counts from K562 cultures treated with 1 mM sodium butyrate (●) and from control cultures without inducer (○). Each point represents the average from duplicate determinations. *b*, Synthesis of haemoglobin in sodium butyrate induced K562 cells as determined by radioimmunoassay. K562 cells were cultivated at an initial density of 0.2×10^6 cells per ml in the presence of 1 mM sodium butyrate (●) or without inducer (○) (the same cultures as in *a*) and aliquots taken on indicated days. After washing the cells three times in phosphate-buffered saline, aliquots were removed for protein determination¹⁵. The rest of the samples were solubilised in Triton X-100 containing buffer and used for radioimmunoassay. Anti-haemoglobin antiserum was prepared in rabbits and anti-rabbit IgG in sheep. Haemoglobin was radioiodinated with ¹²⁵I by the chloramine T method¹⁶. The antibody concentration was calibrated to bind approximately 30% of the radioactivity and more than 95% of the binding was inhibited by nonradioactive haemoglobin. The preparation of antisera and the radioimmunoassay will be described in detail elsewhere. The results are given as µg haemoglobin per g of protein and each point represents the average of duplicate analyses.

As the cultures of K562 cells were found to contain occasional benzidine-positive particles (Table 1) the differentiation capacity of this cell line was further investigated. Because the commonly used inducing agent dimethyl sulphoxide¹ did not cause obvious erythroid differentiation, sodium butyrate was used as inducer¹⁴. When K562 cells were cultivated for 4 days in the presence of 1 mM sodium butyrate (optimal concentration 1–3 mM) differentiation was observed. This amount of sodium butyrate did not significantly affect the growth rate of the K562 cells (Fig. 2*a*). Eosinophilic particles resembling erythrocytes accumulated in the cultures. These particles were surrounded by a membrane and showed positive staining reaction with benzidine (Table 1) indicating the presence of haemoglobin (Fig. 1). The induction of haemoglobin synthesis was confirmed by radioimmunoassay (Fig. 2*b*).

The mode of generation of the erythrocyte-like particles seems to be unusual. Although occasional normoblast-like cells were seen (Fig. 1*e*), most of the erythrocyte-like particles are apparently generated intracytoplasmically (Fig. 1*c*). As shown in Fig. 1*c–e* this seems to start with an accumulation of cytoplasmic eosinophilic inclusions which condense to one or several erythrocyte-like particles which are subsequently expelled. Scanning electron microscopy shows the erythrocyte-like particles to be spherical with a smooth surface resembling spherocytes (in preparation). The mechanism may be similar to the platelet generation by megakaryocytes. The phenomenon of *in vitro* erythrocyte generation by this cell line is being further studied using time-lapse cinematography.

The findings presented here not only prove the erythroid character of the K562 cell line, but also further emphasise that malignant transformation of human haematopoietic cells does not necessarily mean irreversible loss of differentiation capacity. After 8 years in culture *in vitro*, the K562 cell line has retained its ability to react to appropriate stimuli by undergoing erythroid differentiation. The effect of sodium butyrate on human erythroid leukaemic cells might have interesting clinical implications.

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Cleavage site of bacteriophage fd gene II-protein in the origin of viral strand replication

DOUBLE-STRAND DNA replication of small single-stranded bacteriophages such as ΦX174 and filamentous phage fd is initiated by highly specific endonucleases. This was demonstrated *in vitro* with the isolation of ΦX *cisA* protein¹ and more recently with that of fd gene II-protein². Both endonucleases produce one single-strand break in the viral strand of superhelical RFI providing an origin for double-strand (ds) DNA replication^{3,4}. For gene II-protein the strand specificity of nicking was demonstrated by cleavage of asymmetrically labelled fd RFI (viral strand ³²P-labelled, complementary strand ³H-labelled) and subsequent alkaline sucrose sedimentation. As a result the viral strand was cleaved into a full length linear form, whereas the complementary strand remained circular (T.F.M. and K.G., submitted). Here we report the position of the nick in the nucleotide sequence of the fd genome.

Gene II-protein was purified from *Escherichia coli* cells infected with phage fd mutants in gene V using the stimulation of DNA synthesis on fd RFI as an assay. The purification steps were ammonium sulphate precipitation and various columns. Details of the isolation procedure will be published elsewhere (T.F.M. and K.G., submitted). The pure enzyme has a molecular weight of 46,000 on SDS gels and under non-denaturing conditions which agrees with the size of gene II-protein from membranous cells structures. The protein was not found in cells infected with phage mutants in gene II.

Superhelical fd RFI DNA was incubated with purified gene II-protein as described in the legend to Fig. 1 resulting in the open circular form (RFII₀). (Phage fd RFII₀ is defined as the cleavage product of fd RFI by gene II-protein.) To determine the exact position of the nick RFII₀ was cleaved with restriction

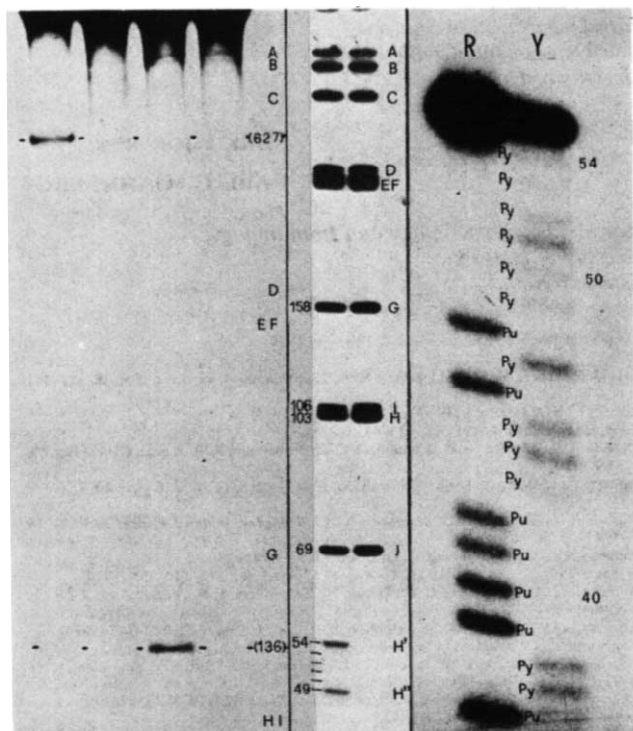


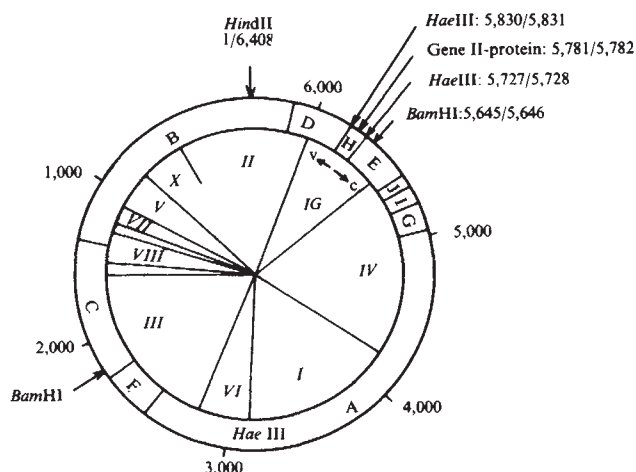
Fig. 1 Localisation of the gene II-protein cleavage site on the fd genome by gel electrophoresis of labelled DNA fragments. The cleavage reaction was carried out with 0.5 pmol (2 µg) fd RFI and 0.3 units of purified fd gene II-protein (T.F.M. and K.G., submitted) in 150 µl 10% sorbitol, 20 mM Tris-HCl, pH 8.1, 80 mM NaCl, 5 mM β-mercaptoethanol, 5 mM MgCl₂ and 200 µg bovine serum albumin per ml for 10 min at 30°C. The reaction product was then digested with restriction endonucleases *Hind*II (tracks a, b), *Bam*HI (c, d), or *Hae*III (e, f). The DNA was denatured in the presence of 5 µg fd single-stranded DNA in 0.2 M NaOH for 20 min at 37°C. The sample was mixed with 200 µl phenol and 50 µl 1 M KH₂PO₄, the aqueous phase desalted on a Sephadex G50 column and lyophilised. Tracks b, d and f are controls without gene II-protein. Labelling at the 3'-end (a-d) was done with nucleotidyl transferase (EC 2.7.7.31) (5 units) in 1 mM CoCl₂, 5 mM MgCl₂, 50 mM imidazol-HCl, pH 6.8 and 1.2 µM [α-³²P]dTTP (2 × 10⁶ c.p.m. in the 25 µl assay) for 30 min at 37°C. The reaction products were analysed on a 5% acrylamide gel. Label at the 5'-ends (e, f) was introduced by T4-polynucleotide kinase (EC 2.7.1.78) after pretreatment with alkaline phosphatase (EC 3.1.3.1) and the fragments separated under denaturing conditions on a 10% polyacrylamide gel¹⁰. The numbers in the figure give the nucleotide length of the fragments generated and the letters the separated *Hae*III fragments, which were also a marker for the tracks a and b. Tracks g and h show a 20% sequencing gel¹⁰ covering the 3' terminus of the 54 nucleotide fragment H'. Strong and weak Py bands correspond to C and T residues, respectively. The very strong bands on top are non-degraded 54 nucleotide fragment.

endonucleases (Fig. 1). The generated fragments were denatured in alkali terminally labelled and analysed by electrophoresis on acrylamide gels. When RFII₀ was cleaved with endonuclease *Hind*II, an additional fragment of about 650 nucleotides was found, when cut by enzyme *Bam*HI, a fragment of about 130 nucleotides appeared in comparison with the control experiment using RFI DNA (Fig. 1). These results indicate that the gene II-protein cleavage site maps around position 5,750 between the *Bam*HI site at nucleotides 5,645/5,646 and the *Hind*II site at nucleotide 6,408/1 within the intergenic region of the phage (Fig. 2). For a more precise analysis restriction enzyme *Hae*III was used. Gel analysis of the denatured reaction products showed two new fragments of 54 and 49 nucleotides derived from the 103 nucleotide *Hae*III fragment (Fig. 1). Nucleotide sequences at the 3'-end of the two fragments⁵ revealed the position of the gene II-protein cleavage site in the viral strand between nucleotide 5,781 and 5,782 (Fig. 3). As gene II-protein is a component of fd RF replication with purified proteins⁴, it seems likely that the enzyme creates a start for DNA synthesis. This origin of phage fd viral strand synthesis lies within the segment derived *in vivo* for the start of DNA replication in the related phage f1 (ref. 6).

Cleavage of fd RFI by gene II-protein gives a 5'-phosphate end and a 3'-OH end. The viral strand at the nick can be extended in the 3' direction with nucleotidyl transferase (small fragment in Fig. 1, track c). The 5'-end can be labelled with polynucleotide kinase only after phosphatase treatment (fragment of 49 nucleotides in Fig. 1, track e). Labelling was as efficient as at the *Hae*III created 5'-end of the adjacent fragment H'. Thus, gene II-protein is not covalently bound to the DNA ends at the nick after alkaline treatment, but it may well be attached to the 5'-end in native conditions. A covalent linkage was postulated for ΦX174 *cisA* protein at the 5'-end of the cleaved viral strand, as label could not be introduced at this end⁷.

The position of the nick between nucleotides 5,781 and 5,782 has several consequences for phage DNA replication and recombination (see Fig. 3). (1) The cleavage site is separated from the origin of complementary strand synthesis⁸ by 24 nucleotides. The complementary strand can therefore only be initiated on the displaced viral strand after a full round of viral strand synthesis. (2) The nick lies in a nucleotide sequence with

Fig. 2 Physical and genetic map of the bacteriophage fd genome. The inner segments represent the genes and the intergenic region (IG) of the phage. The outer segments give the fragments produced by restriction endonuclease *Hae*III. The arrows at the outside indicate the cleavage sites of restriction endonucleases *Hind*II and *Bam*HI, and of gene II-protein, respectively. The arrows in the IG region indicate start and direction of viral strand (v) and complementary strand (c) synthesis. Counterclockwise movement on the map corresponds to the 5' → 3' direction of the viral strand DNA. The numbers at the outside indicate the nucleotide position on the genome.



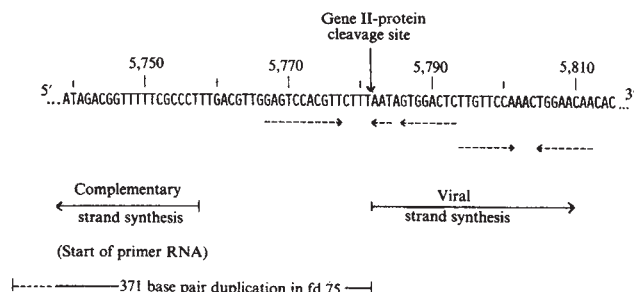


Fig. 3 Nucleotide sequence of the origins of phage fd replication. The interrupted arrows indicate sequences of twofold symmetry around the cleavage site of gene II-protein, which may be in equilibrium with a hairpin in single-stranded viral DNA and in superhelical RF DNA. Viral and complementary strand synthesis start closely neighbored but without overlap. In the larger phage fd 75 (H. F. Lauppe and U. Hess, unpublished) a tandem duplication of base pairs reaches from the gene II-protein cleavage site into gene IV (ref. 9).

twofold symmetries (Fig. 3). As gene II-protein neither reacts with fd single strands nor with relaxed doubly closed fd RF (T.F.M. and K.G., submitted), we assume that transient hairpin structures in the locally disturbed DNA double helix are recognised by the enzyme. The self-annealing structure may also be required for circularisation of linear single strands in RF and viral strand synthesis. (3) The nick in the RF also represents a hot spot for recombination with homologous sequences in other parts of the genome which result in duplications of DNA segments found in variants of filamentous bacteriophages⁹.

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Premature termination during adenovirus transcription

INITIATION of transcription, the first event in gene expression, is probably the most critical possible site for gene regulation. However, it has become clear from recent studies with bacteria and bacteriophages that a transcriptional start does not ensure the expression of a gene. For example, the phenomenon of 'attenuation', that is, premature termination of an mRNA chain before transcription of the structural gene region, is a key regulatory event for the synthesis of enzymes that make amino acids in bacteria^{1–4}. We report here evidence for premature chain termination during transcription of an animal virus genome.

Late in adenovirus type 2 (Ad2) infection of HeLa cells a large group of virus-specific mRNAs^{5–7} are produced by processing

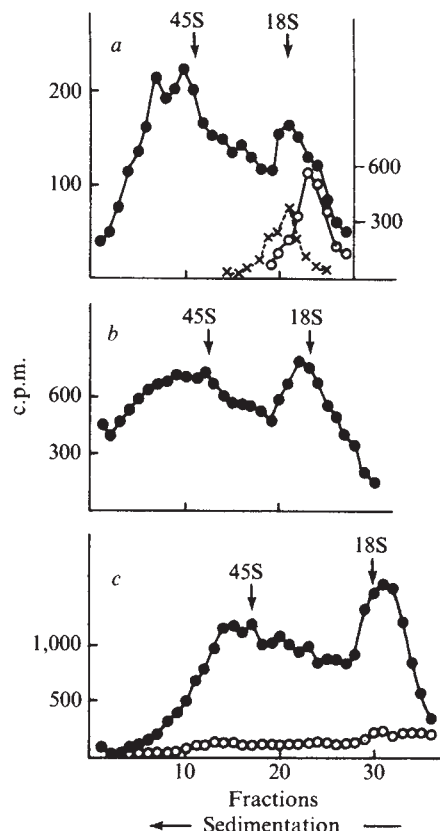


Fig. 1 Sedimentation analysis of 'nascent' Ad2-specific nuclear RNA. *a, b*, Labelled nuclear RNA was extracted from Ad2-infected HeLa cells after a 45-s label with ³H-uridine. 1–2 × 10⁸ cells were collected 16–18 h after infection, resuspended at 4 × 10⁶ cells ml⁻¹ with 1 mCi ³H-uridine ml⁻¹, final concentration 0.05 μmol uridine ml⁻¹; after 45 s the cells were poured over frozen crushed medium. The extracted RNA was treated with dimethyl sulphoxide at 65 °C as described previously^{8,17} and sedimented at 25 °C in sucrose SDS gradients. Markers were either A₂₆₀ furnished by ribosomal RNA or ³²P-labelled ribosomal RNAs. The positions of 45S and 18S are indicated. Fractions of the gradients equivalent to 10⁷ cells were hybridised²⁷ to 2 γ of Ad2 DNA adsorbed to nitrocellulose filters (●). RNA:DNA hybridisation was carried out as described at 65 °C in 2 × TES buffer containing 0.3 M NaCl, 0.01 M TESS, 0.01 M EDTA and 0.2% SDS. Hybridised RNA resistant to ribonucleases A and T was scored. In panel A samples equivalent to 3 × 10⁷ cells were hybridised to 2 μg equivalent of Sma1 B, 18.2–36.7, (×) and Sma1 F, 11.1–18.2 (○) per filter. *c*, Nuclei from cells infected with Ad2 for 19 h were isolated^{8,9} and labelled for 2 min with ³H-UTP either with or without 0.5 μg α-amanitin. Nuclear RNA was extracted, sedimented and hybridised to total Ad2.

the primary transcripts from a single large rightward reading transcription unit^{8–14}. The location of the promoter site for this large transcript at 16.45 on the Ad2 genome^{9,15} makes an assay for the frequency of transcription of the beginning of this transcription unit compared to the transcription of the remainder of the region possible. (The linear Ad2 genome is divided into 100 units of 355 bases each with 0 at the left end of the DNA strand that is transcribed to the right.) This assay utilises labelled 'nascent' RNA (unfinished chains labelled only at their growing ends^{8,9,16,17}) hybridised to appropriate Ad2 fragments produced by restriction endonuclease digestion. From the present experiments it appears that as many as 70–80% of the RNA polymerase molecules that initiate Ad2 RNA chains at 16.45 fail to continue for more than ~2,000 nucleotides. The polymerases that are responsible for producing the primary transcripts from which the majority of the late Ad2 mRNA is derived continue to ~98.5, a distance of ~29 kilobases^{8,9,14–16,18,19}.

If cells or isolated nuclei are exposed very briefly to labelled RNA precursors, the labelled RNA chains from a single transcription unit will form an equimolar series of nascent chains labelled at their growing 3' termini^{8,9,16,17}. All chain lengths between very short polynucleotides and the finished length should be equally labelled if no premature termination and no processing occurred during transcription. Figure 1c shows that Ad2-specific RNA labelled *in vitro* in nuclei isolated from Ad2 infected cells presented a bimodal distribution of Ad2-specific chains. A broad continuous distribution of Ad2-specific RNA from about 2–20 kilobases was observed with a peak sedimenting faster than 45S pre-ribosomal RNA (14 kilobases). Also, a distinct peak of shorter Ad2-specific RNA that sedimented about equally to 18S (~2 kilobase) rRNA was observed. This result repeats that of an earlier experiment⁸, where it was further shown that the 10–18S *in vitro* labelled Ad2-specific RNA was complementary mainly to DNA fragments 11.1–18.2 and 18.2–36.7, that is, to regions near the start site (16.45) for late Ad2 RNA synthesis. To test whether the same pattern of transcription also occurred *in vivo*, 'late' Ad2-infected HeLa cells were labelled with ³H-uridine for 45 s. The nuclear RNA was isolated and separated by sedimentation and the various sized RNA chains were hybridised to Ad2 DNA. Two experiments are shown in Fig. 1a, b. In both a bimodal distribution of large (~45S pre-rRNA) and small (~18S and less) Ad2-specific RNA sequences was observed. In approximately five *in vivo* experiments the sedimentation pattern of Ad2-specific RNA was similar. The amount of 'short' Ad2-specific RNA varied from a peak about one-half as great as the large RNA to approximately twice as great as the large Ad2 RNA.

To determine the origin of the short *in vivo* labelled Ad2-specific RNA the 6–18S region from one *in vivo* labelled preparation was pooled and hybridised to a series of fragments of the Ad2 genome prepared by restriction endonuclease digestion (Table 1). Over 75% of the smaller Ad2-specific chains hybridised to DNA fragments 11.1–18.2 and 18.2–36.7 that contain the first part of the transcription unit, in accord with the *in vitro* result^{8,9,15}. Most of the remainder of the small RNA hybridised to the 3–11 region where two or three independent transcription units are known to exist^{9,20,21}. If every polymerase which started at ~16 continued through the entire major late transcription unit (to a terminus near ~99 (ref. 19)) the distribution of labelled chain lengths would be unimodal in a sucrose gradient. The bimodal distribution with an enrichment

Table 1 Hybridisation to Ad2 DNA restriction fragments of small (10–18S) *in vitro* labelled RNA

Coordinate of DNA fragment	c.p.m. hybridised	% Total
3. –10.7	185	5%
11.1–18.2	897	25%
18.2–36.7	1,963	53%
36.7–40.5	66	
40.5–52.6	85	
58.5–70.7	72	
70.7–75.9	90	
75.9–83.4	33	
83.4–89.7	70	

³H-uridine labelled RNA of ~6–18S (equivalent to fractions 21–27 in Fig. 1a, b) was collected from cells that were pulse-labelled (45 s) at 18 h post-infection (see Fig. 1). The input was equal to the 6–18S RNA from 2×10^7 cells. Three successive rounds of hybridisation were carried out to DNA filters containing 10 µg equivalent of each fragment (that amount of a given fragment that would be contained in 10 µg of total Ad2 DNA). The hybridisation was for 48 h at 65 °C in $2 \times$ TES (0.01 M N-tris(hydroxymethyl)methyl-2-aminoethane sulphonic acid, 0.01 M EDTA, 0.2% SDS)²⁷. After the first round of hybridisation the supernatant solution was withdrawn and the filters were washed twice at 65 °C with $2 \times$ SSC (standard saline citrate; 0.15 M NaCl, 0.01 M Na citrate). All the unbound RNA was collected by precipitation with ethanol and the second round of hybridisation was carried out. The third round was then performed. Approximately 60% of the hybrids formed in the first round, 30% in the second round, and 10% in the third round.

of sequences complementary to the promoter proximal regions of the transcription unit is evidence that premature RNA chain termination occurs after about 1,000–2,000 nucleotides both *in vitro* and *in vivo*.

To determine the frequency of premature termination the amount of RNA synthesised in 45 s that was complementary to a variety of DNA fragments was measured. The amount of labelled RNA complementary to any DNA fragment divided by the length of the fragment that is transcribed is a measure of the 'molar' amount of RNA synthesised from that DNA region^{8,9,14}. The length of the 11.1–18.2 region that is transcribed was taken to be 600 nucleotides, from 16.45 to 18.2, the distance from the presumed promoter to the end of this fragment¹⁵. All of the other fragments except the terminal fragments 89.7–100 were

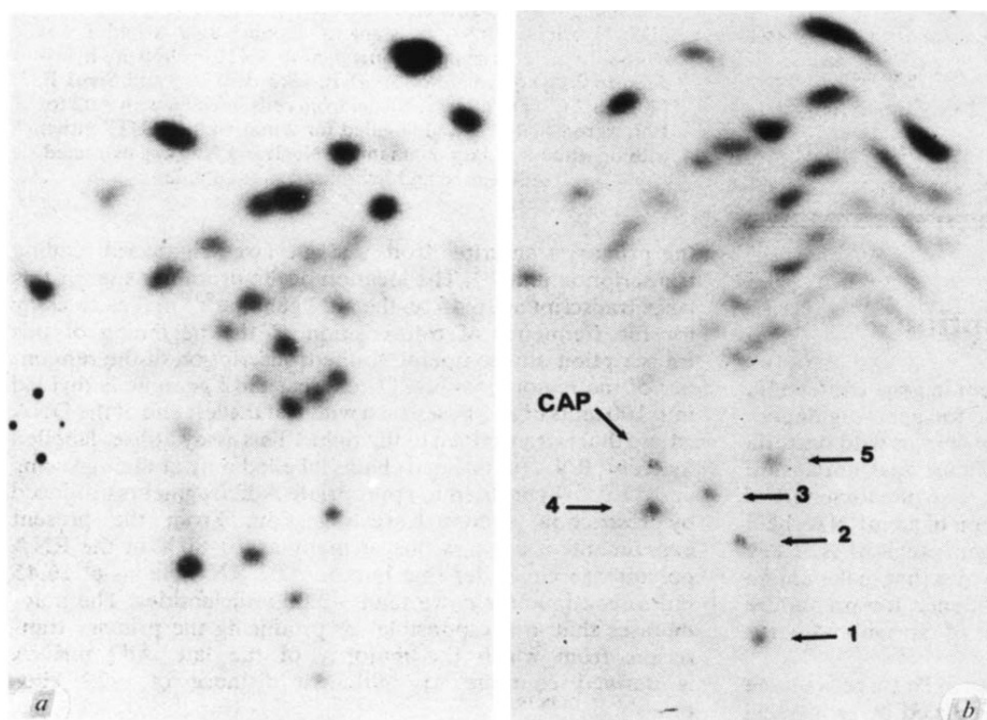


Fig. 2 Two-dimensional oligonucleotide analysis of RNA hybridised to the 11.1–18.2 region of Ad2 DNA. Ad2-infected HeLa cells were labelled with ³²P (2×10^8 cells were exposed to 50 mCi carrier-free ³²P-orthophosphate 14–18 h after infection) or nuclei from infected cells were prepared 18 h post-infection and labelled by *in vitro* incorporation of [α -³²P]UTP for 2 min⁸. Nuclear RNA was extracted from both samples and the *in vivo*-labelled RNA >28S was selected by sedimentation. The RNA was hybridised to the Sma1 F DNA fragment 11.1–18.2 using 100-µg equivalent filters per 10^8 cell equivalents, eluted after T1 digestion and extensive washing and fingerprinted as described previously¹⁵. Oligonucleotides labelled 1–5 were eluted and secondary analyses (pancreatic digestion followed by DEAE electrophoresis)¹⁵ confirmed that the same sequences were present in the *in vivo* and *in vitro* labelled oligonucleotides. For the *in vitro* labelled sample the extramolarity for Sma1 F, 11.1–18.2, was demonstrated as in Table 2 and 95% of the Sma1 F-specific incorporation was inhibited by α -amanitin.

Table 2 Molarity of transcription of regions of Ad2 genome

DNA fragments	Length transcribed* (kilobases)	Expt 1		Expt 2		Expt 3		Expt 4	
		c.p.m. in hybrid	Molarity†	c.p.m. in hybrid	Molarity	c.p.m. in hybrid	Molarity	c.p.m. in hybrid	Molarity
11.3–18.2	0.6	1,319	6.7	1,385	6.8	521	6.4	2,678	3.3
29.1–36.7	7.5	—	—	—	—	990	0.98	—	—
29.1–52.6	3.4	—	—	—	—	—	—	3,227	0.71
36.7–40.5	1.1	381	1.05	395	1.1	—	—	—	—
40.5–52.6	4.4	940	0.65	1,239	0.84	—	—	—	—
58.5–70.7	4.3	1,240	0.86	1,104	0.75	493	0.85	3,256	0.6
75.9–83.4	2.8	460	0.51	500	0.55	—	—	—	—
83.4–89.7	2.0	655	1.0	672	1.0	270	1.0	2,685	1.0
89.7–100	3.2	—	—	—	—	290	0.65	3,121	0.72

³H-uridine labelled nuclear RNA was prepared after a 45-s exposure of Ad2 infected HeLa cells (16–18 h post infection) and the total RNA sample was hybridised as indicated to various Ad2 DNA restriction fragments (see Fig. 1 for details). Two or three rounds of hybridisation were carried out in each case.

* The length transcribed, in kilobases, was taken to be the whole fragment except for the 11.1–18.2, *Sma*I F fragment where only 0.6 kilobases from 16.45–18.1 is transcribed^{8,14} and for the 89.7–100 *Eco*C fragment. It is probable that the termination site for transcription is at ~98.5 (ref. 19) and the DNA transcribed in this fragment would be 3.2 kilobases.

† Molarity was calculated by dividing the c.p.m. in hybrid by the length transcribed for each fragment and normalising to 1.0 for 83.4–89.7 fragment which was used in every experiment.

assumed to be completely transcribed. Transcription of the terminal fragment was taken to be 89.7–98.5 (ref. 19). When the molar amount of synthesis from each DNA region was calculated, there was three- to sixfold more labelled RNA complementary to 16.45–18.2 than to any other DNA fragment (Table 2). The remainder of the fragments were essentially equally transcribed, a result which has been obtained in other experiments^{8,14,19}.

In calculating the molarity of transcription from the 11.1–18.2 region an assumption was made that all transcription reads rightward beginning at 16.45. This assumption is justified from previous experiments²¹ that showed 98.5% of total late transcription to be rightward reading. In addition, other recent experiments by Sehgal *et al.*²² showed that 90% of the nuclear RNA labelled in a 2-min pulse that was complementary to the 11.1–18.2 region was complementary to the *r* strand.

Evidence that the short and presumably prematurely terminated RNA contains the same sequences and thus begins at the same site as those molecules that eventually give rise to mRNA is given in Fig. 2. Infected cells were labelled with ³²PO₄³⁻ and nuclear RNA from molecules larger than 5,000 nucleotides was hybridised to the 11.1–18.2 fragment and unpaired RNA digested with T1 RNase. The filters were then washed extensively and the RNA eluted, digested with RNase T1 and fingerprinted. The hybridised RNA (Fig. 2a) revealed the characteristic large oligonucleotides that were previously shown to be near the 5' end of long nuclear RNA transcripts¹⁵. These T1 oligonucleotides were all predicted on the basis of direct sequence analysis to be part of rightward reading RNA molecules beginning at 16.45. As previously reported no oligonucleotides characteristic of sequences to the left of 16.45 were observed¹⁵. For comparison, isolated nuclei which continue synthesis of chains already initiated⁸ and which exhibit premature termination (Fig. 1c) were labelled with [α -³²P]triphosphates, and RNA complementary to 11.1–18.2 isolated. A fivefold molar excess of the *in vitro* labelled ³²P-labelled RNA to the 11.2–18.1 region was detected by hybridisation of a small portion of the preparation to a series of DNA fragments as had been done in the experiments of Table 2 (data not shown). When this ³²P-labelled RNA was bound and eluted from the 11.1–18.2 DNA, subjected to T1 digestion and analysed by fingerprinting, the same characteristic large oligonucleotides as those observed in the *in vivo* ³²P-labelled RNA were most prominent. In addition, many smaller oligonucleotides coincided (Fig. 2). Thus the prematurely terminated chains appeared to start at the same site as the chains that eventually are processed into mRNA. It is not possible, of course, from this experiment to be certain that the same exact 5' end is present on the prematurely terminated

RNA. However, preliminary evidence from *in vivo* labelled RNA (Fraser, Sehgal and J.E.D., unpublished) indicates that the short Ad2 chains that accumulate in a long ³²P label in infected cells have a similar fingerprint as that of Fig. 2 and contain the same capped oligonucleotide characteristic of the large late transcription unit.

Thus, the major result of the present experiments is that three to six times as much transcription occurs, presumably beginning at 16.45, as in other regions of the large late transcription unit. The sucrose sedimentation of pulse-labelled RNA (Fig. 1a, b) suggested that the extra transcription could continue as far as 2,000 nucleotides and this conclusion was also supported by the hybridisation of the slower sedimenting second peak of Ad2-specific RNA mainly to the 11.1–18.2 and 18.2–36.7 DNA fragments (Table 1 and Fig. 1a). From the present data we cannot conclude whether there are specific termination sites. The extra-molar synthesis does not extend past 25.5–27.9 because pulse-labelled RNA complementary to this region was earlier shown to be equimolar with all other regions to the right of 25 (refs. 8, 14). Thus the third of the leader sequences at 26.7 that is spliced to late Ad2 mRNAs^{11–13} does not appear to be represented in the extra synthesis, although each late mRNA is believed to contain all three leader sequences^{6,11–13}. Furthermore, it seems very unlikely that the prematurely terminated chains lead to production of mRNA molecules containing sequences that lie exclusively within the first 2,000 nucleotides of the transcription unit. Extensive searches for mRNA:DNA duplexes by electron microscopy^{6,11} have not revealed mRNA that lies within the 16–25 region and fingerprint analysis of cytoplasmic RNA does not show the distinctive oligonucleotides of Fig. 2 which lie within the first 500 nucleotides of the transcription unit¹⁵. Thus very few if any of the extra-molar premature transcripts appear as stable mRNA in the cytoplasm.

One final point concerns the nature of the enzyme responsible for the short prematurely terminated RNA chains. In the experiments of Figs 1 and 2, a portion of the isolated nuclei during incorporation of labelled precursor was treated with 0.5–1.0 μ g ml⁻¹ of α -amanitin, a dose of this inhibitor that almost completely inhibits synthesis by RNA polymerase II but not RNA polymerases I or III (ref. 23). The synthesis of >90% of the Ad2-specific RNA, both short chains and long chains, was sensitive to α -amanitin (Fig. 1c, legend to Fig. 2). Therefore, it seems that it is RNA polymerase II that prematurely terminates RNA chains within the first 2,000 nucleotides of the large Ad2 transcription unit. Other recent studies have shown that polymerase II transcription termination also frequently occurs in cell heterogeneous nuclear RNA formation (ref. 26, M. Salditt-Georgieff, M. Harpold and J. E. D., in preparation).

Transcription termination as a regulatory mechanism has been extensively documented in the temperate bacteriophages^{24,25} in addition to the attenuation phenomenon in the amino acid operons in *Escherichia coli*¹⁻⁴. In these instances the frequency of termination is very high, occurs on a region immediately preceding structural gene clusters and probably involves interactions between ribosomes translating the new 5' terminus of the RNA chain and the polymerase molecule that is synthesising the RNA chain. If there is any regulatory role at all for premature termination on mammalian cell or Ad2 DNA it would be unlikely to involve ribosomal interaction with growing chains of RNA in the nucleus of a mammalian cell and so no mechanism of premature termination parallel to bacterial attenuation seems likely. A cause for premature RNA chain termination other than a direct regulatory role might be found in reports about the complexity and steps of Ad2 mRNA formation¹⁴. Based on the close linkage in time between transcription, selection of a site and addition of poly(A), polymerase II may be accompanied by processing enzymes or may possess processing subunits¹⁴; possibly if polymerase-processing complexes that initiate transcription are incomplete they may be rejected after a certain distance from the origin of transcription.

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Sequence of three introns in the chick ovalbumin gene

WE now know that many genes are split, that is, that the DNA is not co-linear with its RNA, but is interrupted by regions of DNA about 10-1,000 residues long which have been called intervening sequences or introns¹. This organisation poses special problems for the biosynthesis and processing of mRNA², but is widespread in occurrence³⁻¹⁷, although some genes are known not to be split^{18,19}. Little is known of the detailed sequence of introns. In the case of globin and ovalbumin genes, hybridisation experiments^{20,21} indicate an absence of extensive sequence homology with other unrelated genes. Detailed sequence analysis of the β -globin genes in mice and rabbits²² suggest that during evolution introns diverged in sequence and in length

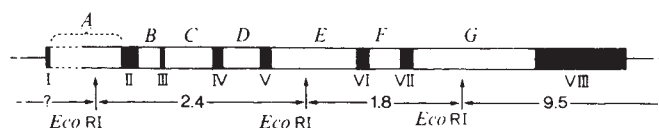


Fig. 1 Arrangement of introns (light, A-G) and exons (dark, I-VIII) in the chick ovalbumin gene. The positions of the cloned 1.8, 2.4 and 9.5 kilobase fragments separated by *EcoRI* restriction sites are shown. The 5' *EcoRI* fragment containing exon I has not yet been characterised so that the length of A is unknown.

more rapidly than the expressed sequences or exons, although the position of the exon-intron junction is precisely maintained²². The ovalbumin gene is a particularly extreme example of an interrupted gene. It has seven introns, and is spread over a distance of at least 6 kilobases (Fig. 1), but this distance is compressed at least 7 times into less than 800 residues of mRNA sequence; all seven known splice points occur between residues 47 and 830 in the 5' half of the ovalbumin mRNA^{23,24}. This gene is thus a favourable model gene for studying both the general properties of introns and the mechanism of splicing. Previously we and others sequenced selected short regions in two cloned fragments (the 1.8 and 2.4 kilobase fragments) of the gene to define the possible recognition site of the presumed splicing enzyme^{24,25}. We now report the sequence analysis of three introns of the 2.4-kilobase fragment of the chick ovalbumin gene.

A clone of the 2.4-kilobase ovalbumin fragment (pOv2.4) contained within the plasmid pBr322 and the vector *Escherichia coli* had been previously isolated and the major restriction sites mapped^{23,26}. After transfer of the pOv2.4 to *E. coli* RRI suitable restriction fragments were prepared which were labelled either at their 5' or 3' ends with ³²P for sequencing by the chemical degradation method of Maxam and Gilbert²⁷. Figure 2 summarises the sequence evidence and the legend gives details of some technical improvements in the methods. It can be seen that most of the sequence was read off both strands of DNA to ensure 100% sequence accuracy. Where data from one strand were

Table 1 Known restriction sites in the 2.4-kilobase fragment

Enzyme	Residue nos	Sequence†
<i>Pst</i> I	1,966	CTGCA/G
<i>Hae</i> III	623	CG/CC
<i>Hin</i> II	766, 968, 1,063	G/ANTC
<i>Hph</i> I	317, 489, 806, 1,425, 1,656, 2,058	TCACC and GGTGA
<i>Alu</i> I	8, 374, 432, 579, 661, 717, 785, 860	AG/CT
	895, 1,055, 1,119, 1,544, 1,727, 1,792, 1,879, 1,964, 1,984, 2,085, 2,111, 2,121	
<i>Mbo</i> II	404, 983, 1,179, 1,397, 1,478, 1,678	TCTTC and GAAGA
<i>Eco</i> RII*	450, 470, 762, 1,102, 1,996	CC(A/T)GG
<i>Sau</i> 3A		
(<i>Mbo</i> I*)	1,109, 1,521, 1,971	/GATC
<i>Xba</i> I	300	T/CTAGA
<i>Sst</i> I	373, 1,983	GAGCT/C
<i>Pvu</i> II	1,963	CAG/CTC
<i>Asu</i> I	1,215	G/GNCC
<i>Mn</i> I	33, 129, 585, 620, 809, 830, 881, 1,018, 1,167, 1,414, 1,634, 1,933, 1,936, 2,158, 2,238, 2,270, 2,285, 2,306, 2,314, 2,332	CCTC and GAGG
<i>Hpa</i> I	181	GTT/AAC
<i>Hin</i> II		
<i>Kpn</i> I	2,240	GGTAC/C
<i>Hin</i> III	2,110	A/AGCTT

There are no sites for *Hae*II, *Hha*I, *Hpa*II, *Hga*I, *Ava*I, *Bam*HI, *Bgl*II, *Sma*I, *Sal*I, *Taq*I and *Xho*I. *Eco*RI sites define the two ends of the fragment. The residue number given is the first (5') nucleotide of the recognition sequence and not the point of cutting. Where this is within the recognition sequence it is indicated by a vertical bar.

* These enzymes fail to cut pOv2.4 grown in *E. coli* strain RRI because of methylation of C(*Eco*RII) or A(*Mbo*I) residues (see text).

† See Roberts³².

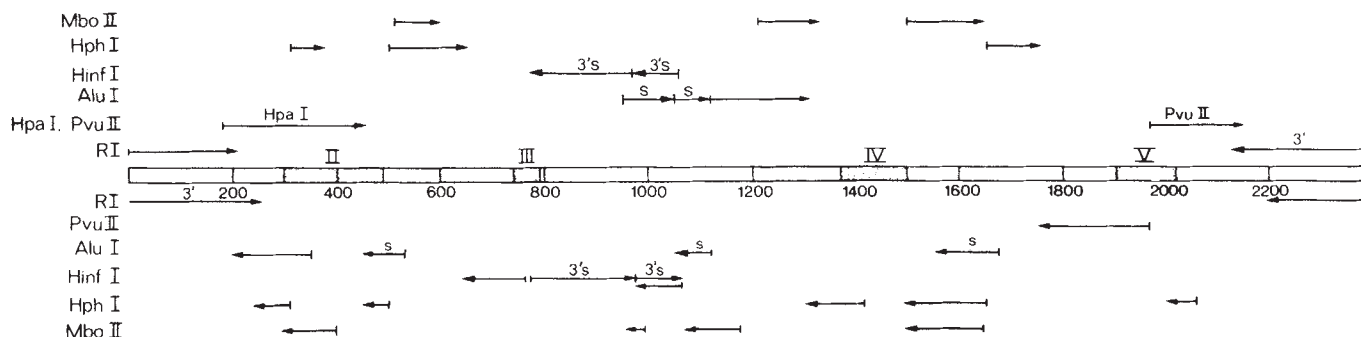


Fig. 2 Diagram of gel evidence showing the overlapping data used to derive the sequence of the 2.4-kilobase fragment (represented by the coding strand 5'-3', left to right). Lines indicate the length of sequence read and the restriction enzyme used to prepare the fragment. Above, the sequence was of the coding DNA strand; below, the sequence of the complementary non-coding strand. Arrows point away from the labelled position. 5'-labelling with ^{32}P was carried out with $[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ and T4 polynucleotide kinase²⁸ using restriction fragments derived by *EcoRI*, *HpaI*, *PvuII*, *MboII*, *HinfI*, *HphI* and *AluI* digestion of either the purified 2.4-kilobase ovalbumin gene fragment (purified by 3.5% acrylamide gel electrophoresis²⁸) or the intact pOv2.4 plasmid directly, so as to obtain higher yields of labelled fragments. 3'- ^{32}P -labelled, *EcoRI* and *HinfI* labelled fragments were also prepared by 'filling in' with a single $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ using the Klenow fragment of DNA polymerase I (*E. coli*) (F. Sanger and J. Hindley, personal communications). Complementary strands in the size range of 20-200 residues were separated by heat denaturation in formamide and electrophoresis on 20% acrylamide 7 M urea gels (G.G.B., unpublished). Separated strands or labelled fragments, recut by another restriction enzyme, were subjected to chemical modification according to Maxam and Gilbert²⁷ using the alternate G, alternate A, C and C+T degradation and fractionation on an 8% or 20% acrylamide, 7 M urea thin²⁹ gel (0.3 x 200 x 400 mm). Longer (800 mm) thin gels (8%) were used to 'read' sequences 200-300 residues from the site of labelling. Sensitive autoradiography was according to Laskey and Mills³⁰. All the exon sequence was established independently of the mRNA except: (1) 10 residues across the *HinfI* overlap (residue 766) in exon III; (2) 60 residues between the *HphI* site (1,425) and *MboII* site (1,478) in exon IV; (3) 10 residues across the *PvuII* site in exon V. A computer file was kept on the sequence as different restriction fragments were sequenced and this was edited using the SEQEDT program of Staden³¹, as new data became available. The file was also routinely searched for possible restriction sites³¹.

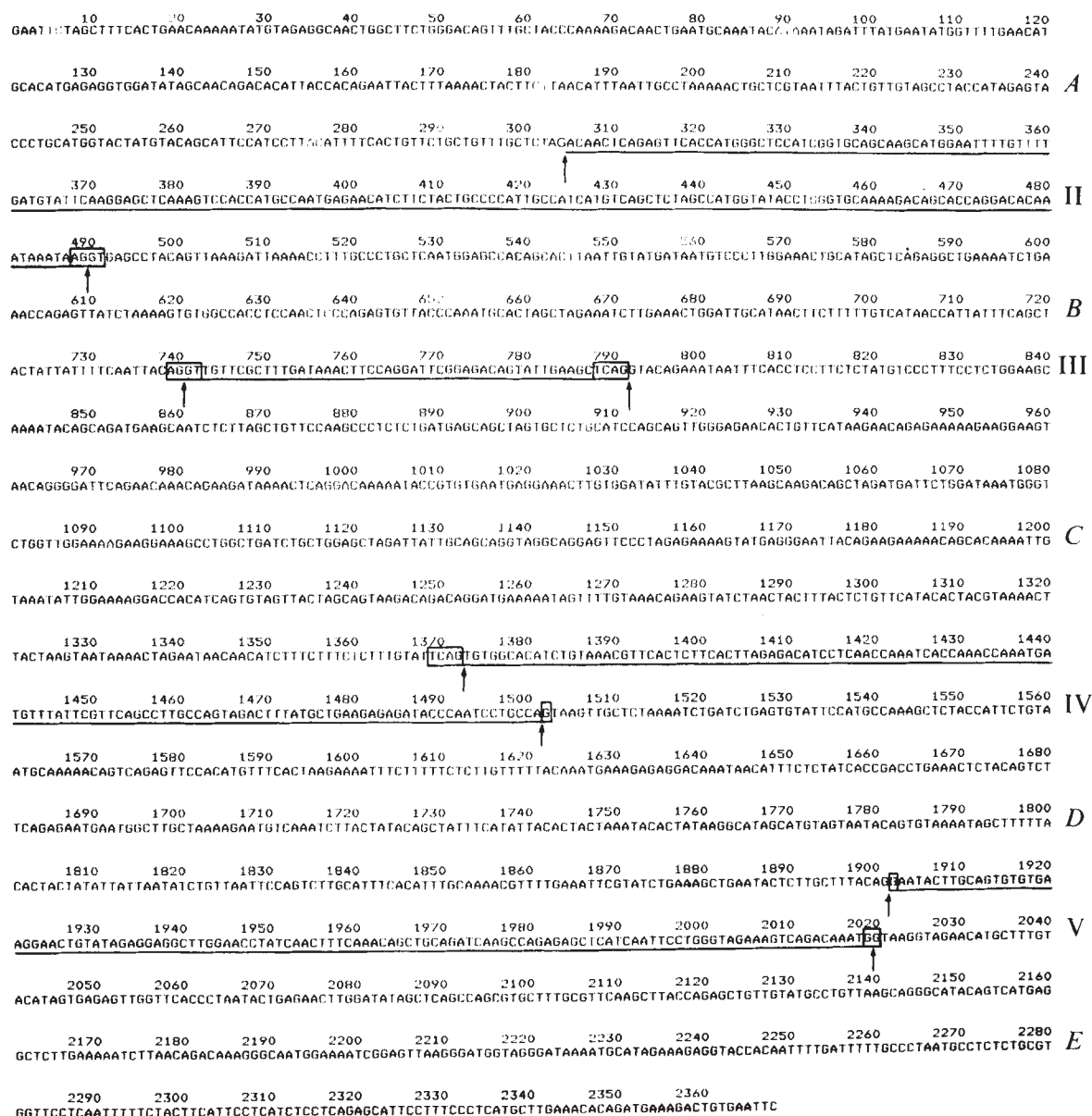


Fig. 3 Sequence of 2.4-kilobase fragment. The exons II-V and introns A-E are identified, as are the boxed regions showing terminal redundancy at each end of the introns. Arrows indicate the predicted splice points (see text).

Table 2 Simple parameters of the 2.4-kilobase sequence

Region (Fig. 3)	Length (no. of residues)	A+T%	CG (frequency)	AA (frequency)
A*	304	63	1	30
B	251	64	0	28
C	581	62	3	81
D	400	68	3	49
E*	349	59	1	31
II	185	54	1	16
III	51	58	2	3
IV	129	57	2	11
V	118	56	0	13

*Introns A and E are incomplete, so that the length and other parameters quoted here reflect only the proportion of these introns present in the 2.4-kilobase fragment.

used, only completely clear gel evidence was used to construct the sequence. A single arrow was invariably constructed from many independent gels.

The restriction enzymes *Mbo*I and *Eco*RII failed to cut the recombinant plasmid grown in *E. coli* RRI because of a methylation of the A residue (in the sequence GATC) or the second C residue (in the sequence CC(A/T)GG (ref. 28)). An anomalous degradation was also present in the sequencing gels, in that 6-methyl A gave a weaker band than an unmodified A in the alkaline degradation (alternate A) procedure²⁷. Similarly, 5-methyl C was resistant to hydrazinolysis, resulting in possible misreading of the gel¹⁹. Therefore, in all such cases of possible ambiguity the sequence was carefully checked on the opposite strand of the DNA.

Figure 3 shows the sequence of the 2,368 residues present in the 2.4-kilobase fragment, and Table 1 gives the position of most of the known restriction sites within this sequence. The exon sequences are underlined in Fig. 3 and were identified by their presence in the mRNA. It codes for 26% of the mRNA, from residue number 48 to 530, out of a total of 1,859 residues²⁸. No differences were found in these regions between the gene and the previously determined mRNA sequence²⁸, although about 80 residues of exon were not established independently (see legend to Fig. 2). Figure 3 indicates the previously noted terminal redundancy at each end of the introns B, C and D and the predicted splice points^{24,25}, either before or after a GT or AG doublet, depending on which end of the intron it is. A general summary of properties such as the length, base composition, expressed as % A+T, and frequency of CG doublets relative to AA doublets are given in Table 2. This shows that the three complete introns (B, C and D) vary by 251–581 residues in length. Exons are remarkably short; the smallest, exon III, is only 51 residues. The entire sequence is A+T rich, but is slightly higher for introns (varying by 62–68%) than for exons (54–58%). CG doublets occur very infrequently (compared with AA doublets) in the exons, as noted before²⁸, and also in all introns. No other doublet behaves like CG.

The main purpose of the sequence analysis of this part of the ovalbumin gene was to focus on introns. We have sequenced three introns completely and in all, nearly 1,900 residues of intron sequence are presented here. We had hoped to find specific primary sequence or secondary structures which might give an insight into the mechanism of RNA splicing. However, despite extensive computer analysis, we have been unable to find any convincing features. We will therefore defer presentation of this analysis until the full structure of the gene is known.

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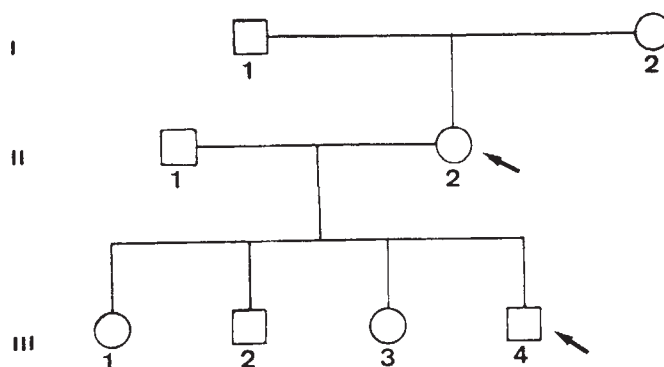
Corrigendum

In the letter 'High frequency recombination in centromeric and histone regions of *Drosophila* genomes' by R. F. Grell, *Nature* **278**, 78–80 (1978), an additional reference should be included following the citation of refs 15 and 16: Goldring, Brutlag & Peacock in *Arrangement of the Highly Repeated DNA of Drosophila melanogaster* (eds Peacock & Brock) 47–59 (Australian National University Press, Canberra, 1975).

Errata

In the article 'Isotopes of tellurium, xenon and krypton in Allende meteorite retain record of nucleosynthesis' by R. V. Ballard *et al.*, *Nature* **277**, 615–620, paragraph 6 line 22 reads: 'Allende's spinels is...'. In paragraph 11, line 56 reads: '... Interference from the (n, 2n) reaction ...'.

In the letter 'Human chimaera detectable only by investigation of her progeny' by W. R. Mayr, V. Pausch and W. Schnedl, *Nature* **277**, 210–211, Fig. 1 was omitted, and is reproduced here.



matters arising

Solar modulation of atmospheric electrification and the Sun-weather relationship

MARKSON¹ has provided a useful summary of reasons for looking to atmospheric electrical structure for an explanation of 'Sun-weather' correlations, but the specific global and local mechanisms which he proposes do not, in the light of available evidence, apparently produce such effects frequently enough to explain the observations. Effects more directly related to magnetospheric and auroral phenomena would seem to show more promise in explaining the observed coupling of solar activity to lower atmosphere electrical phenomena.

The global effect proposed by Markson is that the 'Forbush decrease'² in cosmic ray intensity associated with solar flares results in a decrease of atmospheric ionisation with consequent effects on the 'global circuit'. Measurements made on a high mountain by Reiter³ have shown that the fair-weather electric field and current show 10–30% effects related to solar magnetic sector boundary crossings. Forbush measured decreases of the order of 1% at low latitudes and even at higher altitudes and latitudes, the effects rarely exceed a few per cent.⁴ As Markson points out atmospheric conductivity is expected to vary as the square root of the Forbush decrease, (ionisation rate) it is difficult to see how this mechanism could produce the observed effects, as decreases of the order of 50% in cosmic ray flux at low latitudes would be required.

The local effect proposed by Markson involves the direct penetration of ionising radiation effects to the tops of thunderclouds, (~20 km at mid and high latitudes). While this is perhaps possible in some rare solar proton (PCA) events⁵, such as the large flare of August, 1972, there is little evidence that substantial conductivity variations occur as low as this after more modest events. Yet balloon measurements at high latitudes^{6,7} typically show a 30% increase in the vertical field following geomagnetically disturbed periods. Thus it would seem that we must look for effects more directly related to magnetospheric and auroral processes to explain the reported data. One problem

would seem to be Markson's acceptance of the 'classical picture' of atmospheric electricity, where an 'equipotential surface is considered to exist at about 60 km', which does not allow the penetration of higher altitude electrical effects. The earliest rocket-borne conductivity measurements in 1950 (ref. 8) showed that the classical exponential increase of conductivity did not persist above 40 km, a result attributed to aerosol particle layers which has since been frequently confirmed⁹. Mühleisen⁷ has observed horizontal electric field gradients totalling ~100 kV between high and mid latitudes following magnetic disturbances, and Tyutin¹⁰ in the USSR has reported a 'permanent' mesospheric vertical field of similar magnitude. (We have tentatively confirmed the USSR measurements and suggested that the 'shorting out' of the vertical field by ionisation produced by aurorally related radiation might produce local changes in the lower atmosphere vertical field and also the observed horizontal potential differences, by adding the permanent mesospheric potential to the lower atmosphere circuit at high latitudes¹¹.) These measurements clearly demonstrate the invalidity of the classical picture for studying many possible coupling mechanisms.

Other promising avenues of investigation would seem to be the direct 'mapping' of ionospheric fields to low altitudes¹² and of aurorally produced charge separation¹³ inducing displacement currents and electric fields in the lower atmosphere. Magnetospheric and auroral phenomena would seem to have a chance of explaining the 22-yr 'magnetic cycle' Sun-weather effects cited by Markson, because of the likelihood that they could be influenced by the details of the solar magnetic field structure. An intermediate mesospheric coupling circuit modulating the lower atmosphere circuit would have the attractiveness of providing a mechanism responsive to auroral particles or bremsstrahlung rather than requiring rare high intensity events, and could thereby provide a more prevalent coupling effect due to the much greater frequency of such events.

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MARKSON REPLIES—Hale's objection may be summarised as: (1) he does not believe that the requisite changes in ionisation occur; nor (2) that the classical picture of atmospheric electricity is valid. He is inaccurate in his quotation and citation, and there is some question regarding his interpretation of data and their significance.

I wish to point out the following: conductivity variations occur sufficiently low in the atmosphere and frequently enough to be a significant factor in solar modulation of current flow in the global circuit. Hale refers to relatively small changes in the galactic component of cosmic radiation measured on the ground which are not representative of the much larger variations of ionising radiation in the lower stratosphere. Winckler¹, in summarising his balloon program in which cosmic rays were measured at mid-latitude (Minneapolis) states "Probably the single most significant finding of the entire series (of flights) was the frequent occurrence of intense low energy cosmic rays. They originated from large solar flares but had energy spectrum so steep that the particles were not detected by the extensive network of sea-level cosmic ray monitors established during IGY. The particles were measured directly, however, by means of balloons at altitudes greater than 20 or 25 km". Not only are solar controlled changes in ion production larger and more frequent in the 20–30 km height range compared to near the Earth, they generally are of the opposite sense, increases instead of decreases^{1–3}. Akasofu and Chapman⁴ (Hale's reference) discuss three Forbush decreases in a 16-day period which resulted in a cumulative 20% reduction in cosmic ray rate at the

Earth's surface. During this interval, Winkler's balloons³ recorded 2–10 fold increases in ion production rate at 24 km, half an electrical scale height above 20 km. Such events, if they were over 20 km high thunderstorms, would at least double the current flow from them. There is also a long period variation in cosmic radiation which is inversely correlated with the sunspot cycle⁵. My Fig. 1, averaged data from Neher and Anderson⁶, illustrated that through a solar cycle at 15 km (100 g cm^{-2}) ionisation increases 51% (conductivity 23%) while at 20 km (50 g cm^{-2}) ionisation increases 76% (conductivity 33%). While these data are for high latitude, Fig. 4 in my article⁷ depicts the variation with latitude of ion production at 20 km. At mid-latitude the conductivity variation through a solar cycle is $\sim 18\%$, near the Equator it is $\sim 10\%$. As these are average values, larger variations must occur at times.

Changes in fair-weather electric field intensity (a measure of ionospheric potential) related to solar sector crossings⁸ does not imply that the electric field is controlled through geomagnetic processes. However, thunderstorms (which maintain the ionospheric potential) have been found to vary with solar sector crossings^{9–11}.

Almost all students of atmospheric electricity accept the general premises of the classical global circuit concept because there is strong evidence supporting this view^{12–15}. As fair-weather electric fields vary simultaneously all over the world and correlate with the universal time variation of global thunderstorm activity^{16,17}, even in the polar cap region¹⁸, upper atmosphere generators (which follow local time) can not be the cause of the ionospheric potential. In addition, the latter would not vary simultaneously over large distances¹⁷ unless the ionosphere had the characteristics of an equipotential surface. It is well known that potential differences can exist across the auroral oval; this does not invalidate the classical picture which is necessary to explain the maintenance and characteristics of the Earth's electric field at sub-auroral latitudes, that is over 85% of the Earth's surface (including most of the portion with thunderstorms). Satellites that record 70–200 kV across the auroral oval do not measure significant potential differences between the auroral zone and mid- to low-latitudes (R. G. Roble, personal communication). Magnetospheric electric fields impressed in the polar ionosphere are attenuated to 10% of their high-latitude value by mid-latitude¹⁹.

The proposed mechanism⁷ does not depend on 'Forbush decreases', this is a generic term used to describe decreases in galactic cosmic radiation following some solar flares. Conductivity, of course, does not vary as the square root of Forbush decreases.

Hale erroneously believes the proposed

mechanism requires a 50% decrease in cosmic ray flux at low latitudes although my article discusses effects on thunderstorm currents at all latitudes and does not mention a 50% decrease in cosmic rays. Perhaps he is referring to the increase in ionising radiations causing a 50% decrease in columnar resistance over the 'global generator' that I used for arithmetic simplicity to illustrate the sensitivity of the entire global circuit to conductivity changes within the small volume over thunderstorms.

The average ground level Forbush decrease is 5% (with a maximum of 20%), not the 1% implied by Hale²⁰.

While the August 1972 flare was unusual in having a very high number density of low to moderate energy particles, numerous other flares have had a larger density of particles in the high energy end of the spectrum²¹. The latter particles penetrate deepest into the atmosphere and cause the effects under discussion.

Ionising radiation does not have to reach the tops of thunderclouds to increase the current flow from them to the ionosphere; it only has to lower the total columnar resistance over the clouds. If, however, ionising radiation did reach the cloudtops, cloud electrification might be enhanced²². Increased ionisation following solar flares has been reported in the 15–20 km height range^{2,3,23}.

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Tyutin's rocket measurements²⁴ (evidently similar to ones made by Hale) indicate that the fair-weather field reverses above 10 km. It is surprising that Hale accepts such data which are contrary to fundamental physical principles. As the vertical air–Earth current is unidirectional from the ionosphere to Earth, electric fields at all heights must be of the same sign.

Concerning aerosol layers above 40 km, such conditions would have little effect on atmospheric electricity in the

lower atmosphere unless the columnar resistance through this upper atmospheric region were a significant fraction of the columnar resistance through the lower atmosphere. There are no measurements indicating that such conditions exist.

Hale cites Akasofu and Chapman⁴ as saying that even at high altitudes and latitudes Forbush decreases rarely exceed a few per cent. This statement is not only erroneous^{1–3}, but it does not appear in the book. Hale's ref. 13 is a paper by Cole and Pierce, not by Freier.

An attempt to explain solar-weather relationships as due to ionospheric and magnetospheric electrical processes is understandable, however, these do not seem applicable to the problem. Upper atmospheric currents and particles would introduce energy into the atmosphere orders of magnitude too small to affect weather processes^{25,26}. They would be important to the extent they influenced thunderstorm currents and cloud electrification in the lower atmosphere⁷.

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reviews

Exploration of the Solar System

J. Veverka

The Realm of the Terrestrial Planets. By Z. Kopal. Pp. 223. (The Institute of Physics: Bristol, UK, 1979.) £7.50.

THIS book is described as a completely up-to-date account of recent advances in the exploration of the Solar System. Written by a well known astronomer who has made important contributions to our understanding of the Moon and the Solar System, the book is well illustrated and pleasant to read. Unfortunately, it abounds in factual errors. From the nature of most of these, it is clear that the author is writing about the Solar System based on information available, for the most part, a decade ago. It is certainly not an up-to-date account of our current understanding of the terrestrial planets, and is especially dangerous to the initiated reader who has no way of distinguishing fact from error.

The factual errors are too numerous to list in detail. For example, in one single chapter, which consists of 39 pages, there are more than a dozen serious factual errors, not to mention an equally large number of potentially misleading and inaccurate statements. For instance, there is much confusion about the polar caps of Mars. Even though recent Viking results are alluded to, no distinction is made between the annual CO₂ frost caps which form every year and disappear during spring and summer, and the permanent (or residual) polar caps, which survive through the Martian summer. In fact, on page 114, we find a restatement of the old misconception that the southern polar cap of Mars vanishes completely on occasion during summer. It is now known that, although the annual cap is composed of carbon dioxide, the residual caps consist of vast deposits of frozen water, a fact apparently unknown to the author. He is unaware of the fact that even the annual caps can have substantial thickness in places because we read on page 119 that the snowfields in the Martian polar regions must be "less than a few centimetres deep", although numerous studies in recent years have shown that several metres of frost can be deposited in the polar regions during Martian winter.

On page 124, we find further evidence of a thorough misunderstanding of Viking data. It is stated the

Martian sky appears pink in daytime, but that it is blue near sunset and sunrise. There is no evidence to support such an assertion, or the claim that the atmosphere is dust free after sunrise but becomes dusty as the sun rises higher and convection begins to lift up surface dust. Published analyses of the Viking Lander data by Pollack and others clearly demonstrate that the dust does not settle out of the atmosphere daily, but remains suspended for periods on the order of the Martian year. There is no evidence in the Viking data to substantiate the statement made on page 125 that a significant number of dust particles are stirred and lifted into the atmosphere by diurnal convection.

On the same page, there is further evidence that the author has not kept abreast of Mars studies in the last seven years. He states that:

"While the bright markings were identified correctly with arid desert plains and their colour ascribed to that of ferric oxides long before the Viking Landers provided the final proof, the dark markings were thought to owe their lower reflectivity to moisture and even to vegetation flourishing in the spring and watered by systems of canals, natural or artificial from the melting polar caps. Their seasonal variations were regarded as indications that at least plant life existed on this planetary neighbour of ours. These expectations were destined to total disappointment by the outcome of the Viking missions."

This is an astounding statement! Soon after the first Mariner 9 pictures were analysed in 1971–72, it became clear that there is no vegetation in the dark areas of Mars, and that seasonal variations are due to the redistribution of dust, by Martian winds. Similarly, at about the same time, Earth-based telescopic measurements by McCord and others clearly demonstrated that the dark areas of Mars are dark because they are less oxidised than the bright areas. No one in the last seven years has proposed seriously that the dark areas are dark either because of moisture, or because of vegetation.

There are several indications that Kopal does not believe or is not convinced that the large structures such as Olympus Mons are really volcanoes. Although one could sympathise with such a cautious attitude, it is much more difficult to take kindly unsupported statements such as that the

whole of Olympus Mons is not more than 10 Myr old.

Perhaps the most colossal blunder in the entire chapter involves sinuous channels. Kopal shows a Mariner 9 picture of some faults on Mars which have never been called 'sinuous channels' by anyone, except Kopal, and have never been attributed to erosions by liquid water by anyone. On the following page, he shows an example of similar features on the Moon and writes, "Note the striking similarity between these lunar features and those on Mars as shown in the previous plate. The origin of these features on the Moon has nothing whatever to do with water". Kopal cautions the reader "against jumping to conclusions about erosion caused by flowing water. For whatever may be a merit of such a suggestion as regards to Mars, it certainly would not apply to the Moon". The whole argument is embarrassing. Nowhere does Kopal show a real Martian sinuous channel. Of course, linear faults were not produced by flowing water.

One should not get the impression that the Mars discussion is the only one marred by serious errors. For example, the discussion of the Mars satellites, Phobos and Deimos, covers only five pages, but contains at least four serious errors. The largest crater on Phobos is not 3 km across; Phobos is not the darkest body in the Solar System: grooves on Phobos most likely do not represent the effects of "cosmic abrasion"; and it is certainly not true that such grooves are "also characteristic of certain parts of the Moon".

There is little point in picking apart other chapters in similar detail—most contain similar serious factual errors and misconceptions.

The Realm of the Terrestrial Planets, although a superficially attractive, well illustrated book, is too full of errors to be recommended to any reader, except as a record of what a noted astronomer thinks the inner Solar System might be like, based mostly on data generally available about ten years ago. It could have been a much more important book if it were really based on current information. □

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Koobi Fora hominids

The Fossil Hominids and an Introduction to Their Context, 1968-1974. (Koobi Fora Research Project, Vol. 1). Edited by M. G. Leakey and R. E. Leakey. Pp. 191. (Clarendon/Oxford University Press: Oxford, 1978.) £15.

THIS is the initial volume of a projected series of monographs on interdisciplinary earth sciences and palaeoanthropological researches in the Koobi Fora area, northeastern Turkana basin, Kenya. That combined research effort unquestionably represents one of the most lastingly important and fruitful investigations into the earlier evolutionary history of Hominidae and the natural setting and biotic associations of Plio-Pleistocene times. This volume provides an historical and methodological introduction (R. E. Leakey); followed by chapters on geology, palaeontology, archaeology and fossil hominids.

A summary of the lithostratigraphy, sedimentary environments and palaeogeography of the Kubi Algi and Koobi Fora formations is provided by I. C. Findlater. This chapter sets out the stratigraphical nomenclature now used for these formations, the members of which are defined on the basis of several spatially extensive tephra. Five principal sedimentary environments are recognised, two lacustrine and three alluvial; and their areal relationships are shown diagrammatically. Eight generalised maps depict palaeogeographical settings of the area of successive temporal intervals during the accumulation of the Koobi Fora Formation. Maps show the locations of the principal hominid finds in the major fossiliferous areas; their temporal relationships and environments of deposition are indicated in stratigraphical section and usefully summarised in a table.

An annotated inventory (and listing) of 87 mammal taxa (exclusive of micromammals) in those formations, as well as mollusc assemblages and their concurrent range zones, and notes on other vertebrate groups is given by J. M. Harris. The fauna of the Kubi Algi Formation is not specified, but is stated to overlap in part that of the (lower) portion of the Koobi Fora Formation. The Ileret, Koobi Fora and Kubi Algi sectors have been each subdivided into a series of numbered areas (19 in the former two, 5 in the latter) for purposes of geological mapping and fossil collection. Tephra deposits are widespread, some demonstrably

spatially extensive and useful in correlation. Certain (named) tuffs serve as marker horizons to differentiate and to subdivide the several formations; however, the relationships, spatially and temporally, of many other (numbered) tuffs are uncertain. Three fossil 'collection units' are now recognised for the Kubi Algi Formation, and five such 'units' for the Koobi Fora Formation, the base of each being defined in a particular area by a named or numbered tuff. However, in the Koobi Fora Formation just over half of all fossiliferous areas in the Ileret sector and nearly half of all such areas in the Koobi Fora sector have lithostatigraphically undefined lower boundaries. Thus, the 'collection unit' concept seems to have limited utility at best, as it is an unfortunate mix of both lithostratigraphy and biostratigraphy.

An overview of the stratigraphical and areal situation, depositional environments and associations, and nature of all major archaeological occurrences, and their content and industrial characteristics is provided by G. L. Isaac and J. W. K. Harris. The

remainder of the volume comprises a well-illustrated inventory, with data on finder, provenience and depositional environment (including many microstratigraphic sections) of all fossil hominids (numbering 129) recovered in the seven years of the project, until 1974 (R. E. and M. G. Leakey and A. K. Behrensmeyer), complemented by an appendix of 30 tables of assorted measurements of those specimens. The scale on some hominid illustrations is unfortunately incorrect. Hominid taxonomy is only briefly mentioned (p89); but not many workers personally familiar with this vast corpus of fossil material will agree with all the attributions suggested. Finally, there is a full bibliography (through 1976) of the project.

This is an immensely useful, well executed and well produced volume of major import to all students of human evolution.

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Coastal sedimentation

Coastal Sedimentary Environments. Edited by Richard A. Davis, Jr. (Springer: New York and Berlin, 1978.) \$22; DM44.

THIS book is intended as a student text and to provide a background on coastal environments for geologists, engineers, oceanographers and coastal managers. Being a multi-author text means that there are certain differences of approach between the chapters; however, this is offset by the obvious expertise that has been brought to bear. The emphasis is on geological description of the morphological features and of the processes that have been interpreted largely from changes in the morphology and from the analysis of internal structures. The chapters have a review style and frequently, to provide an explanation satisfactory for students, would necessitate lectures tailored to the book, or the student to follow up extensively the quoted papers. An example of this is the very brief coverage of longshore transport, which in itself would warrant a chapter in a more specialist book. The bibliographies are reasonably comprehensive and there are abundant and very good photographs, especially of tidal deltas.

There are chapters on river deltas by L. D. Wright, which particularly concentrates on the processes in the Mississippi delta, and on coastal bays by R. B. Biggs, which actually emphasises suspended sediment in estuaries. There is a particularly comprehensive description of American East Coast salt marshes by R. W. Frey and P. B. Basan, and coastal dunes are described by V. Goldsmith. R. A. Davis describes the beach and nearshore zone, and mesotidal inlets and estuaries are covered by J. C. Boothroyd. The latter concentrates largely on tidal deltas. The final two chapters are by J. C. Kraft on the coastal stratigraphic sequences produced mainly by transgression, and by W. T. Fox on modelling coastal environments.

Virtually all of the examples quoted are drawn from the American East or Gulf Coasts. Consequently the extensive body of European literature is largely neglected, together with such features as high range tidal estuaries and coasts. Nevertheless, this will be a useful addition to texts on coastal sedimentation and should be recommended reading on courses covering coastal geomorphology and sedimentology, as well as providing useful background in a wider range of disciplines.

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Cloud physics

Microphysics of Clouds and Precipitation. By H. R. Pruppacher and J. D. Klett. Pp.706. (D. Reidel: Dordrecht, The Netherlands, 1978.) Dfl. 85; \$39.

DURING the past decade the major emphasis in cloud physics has been directed away from fundamental microphysical processes—which had hitherto largely dominated the evolution of the subject, and in which progress had been based primarily upon laboratory investigation—to field investigations of larger-scale effects, in which the cloud dynamics are recognised to play a central role. This change of approach has been dictated in part by the development of superior experimental techniques—radar, optical particle samplers, high-speed data processing systems, and so on—and in part by the recognition that the major obstacle to progress has been the absence of comprehensive and reliable observational data. Accordingly, the publication of a textbook devoted exclusively to microphysical processes might appear at first sight to be somewhat anachronistic. In fact, however, I feel that this comprehensive and rigorous critical analysis of current understanding of the physics of the multitude of processes which form the basis of the subject, will be of invaluable assistance to research workers. The classic text in this field for two decades has been Mason's massively authoritative *The Physics of Clouds* (Clarendon Press, Oxford, 1957) but an enormous amount of material has been published since the second edition of this work appeared in 1971, and the small number of generally excellent monographs that have been produced in the intervening years have been insufficiently detailed to take full account of this new work.

The range, tenor and emphasis of the book can readily be appreciated by studying the titles of the chapters which follow the short historical review: microstructure of atmospheric clouds and precipitation (the only chapter concerned predominantly with field observations); the structure of water substance; equilibrium between water vapour, water, aqueous solutions and ice in bulk; surface properties of water substance; equilibrium behaviour of cloud drops and ice particles; homogeneous nucleation; the atmospheric aerosol; heterogeneous nucleation; hydrodynamics of single cloud and precipitation particles; cooling of moist air; mechanics of the atmospheric aerosol; diffusion growth and evaporation of water drops and ice crystals; cloud particle interactions—collision coalescence and breakup; growth of cloud drops by collision and coalescence;

microphysics of ice particle-drop interactions; the electrical state of the atmosphere and its effect on cloud microphysics. In addition, there is a series of useful, mathematically rigorous and sometimes novel appendices, on more specialised topics.

A feature of this book is the consistent attempt to provide a critical appraisal of published work. In general, these analyses are penetrating and balanced. Another impressive aspect is the bibliography which contains about 2,000 papers, concerning research carried out up to 1978.

A disadvantage of the analytical, microphysical approach adopted by the authors is that although individual topics are critically examined a broader overview of the status of areas of cloud physics is not consistently presented.

The emphasis on the physics rather than the dynamics and raw data is a deliberate and defensible policy which, however, is not uniformly successful. For example, the important research leading us towards a solution of the problem of thunderstorm electrification has been in the field—the laboratory and theoretical studies on which the authors have concentrated attention are of secondary consequence. These limitations—if valid—are minor, however, and there is little doubt that this impressive book will be of central importance to cloud physicists and scientists working in related fields.

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Inorganic analysis

Vogel's Textbook of Quantitative Inorganic Analysis. Fourth edition. Revised by J. Bassett, R. C. Denney, G. H. Jeffery and J. Mendham. Pp.925. (Longman: London and New York, 1978.) £14.

This fourth edition of 'Vogel' by four members of the late Dr Vogel's staff is considerably different from the previous versions that served as the standard textbook of quantitative inorganic analysis for several generations of British chemists. It uses SI units throughout, but in line with the recommendations of the Analytical Division of IUPAC it retains the concept of 'the equivalent' and the 'normal' solution, where it is more convenient and less ambiguous so to do. This is sound common sense in an era that spans the old and the new systems of nomenclature, and it serves the needs of a wide range of chemists and of industrial and academic requirements. The authors are to be congratulated on their pragmatic approach which may, of course, be denigrated by the pundits on either side, but which is eminently sensible at the present time.

Inevitably the new edition shows signs of fairly drastic pruning in the area of classical gravimetric and titrimetric analysis in the sense that the number of alternative classical procedures has been considerably reduced. The methods that have been retained are generally, in the reviewer's opinion, those that are most useful both on a practical analytical basis and for the purpose of training the next generation of students how to undertake

the elements of classical analysis that are still much used in industry and are likely to be required for many years to come. The decision to expand the section on sampling procedures is doubly welcome as all too many textbooks ignore this vital function without which most analyses are virtually pointless.

A new chapter has been devoted to thermoanalytical procedures and the one on infrared analysis which featured in the third edition has been deleted. This has allowed considerable expansion in other areas of spectrochemistry, for example, atomic absorption and emission spectroscopy, and a new chapter has been added on gas chromatography. The new arrangement of the material in the section on electroanalytical chemistry is also a considerable improvement on the previous edition. There have also been some changes in the extremely useful appendices that have always characterised Vogel, for example, the omission of 'chemical factors' and the little used five-figure logarithms, though the four-figure tables are still there to help the chemist with his pH calculations.

Taken all round Vogel is still an excellent book and, in this reviewer's opinion at least, the authors have got the balance right. It contains a very useful blend of the best of classical and neo-classical techniques with the basis of many modern instrumental techniques set out in a way that the student can assimilate readily and follow subsequently with the selected list of references and recommendations for more advanced or detailed reading. The balance between theoretical background and laboratory practice is probably much better than it was even in the previous editions. The book is neatly printed and set out, there are

many useful illustrations and not too many 'commercial' pictures and line diagrams. A mine of information? Yes, but also a very interesting and stimulating one for all who have the opportunity to quarry therein. Unquestionably this is one of the best books available in this area. I only wish the

publishers had had more faith in its success to reduce the price and thus allow a greater audience of students to expose themselves to having their own copy.

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Condensed state helium

The Physics of Liquid and Solid Helium. Part II. Edited by K. H. Bennemann and J. B. Ketterson. Pp.750. (Wiley: New York and Chichester, UK, 1978.) £45.85.

THE helium atom probably comes closer than any other to the miniature "billiard ball" of classical kinetic theory. One might expect, therefore, that the physics of liquid and solid helium would be exceptionally simple and even—perhaps, rather dull. The reality is far otherwise, thanks mainly to the profound influence of quantum statistics: a collection of these small, rigid spheres displays a quite unparalleled richness and variety of physical phenomena some of which, such as bulk superfluidity, are unique on earth (although they may, perhaps, also manifest themselves within the nucleonic fluids in the interiors of dense stars).

In the 1960s it was still possible (just) for a single author to prepare a research monograph providing a reasonable coverage of virtually everything that was known about condensed state helium; but, such has been the expansion of knowledge over the intervening years, this is hardly the case any more in the late 1970s. The admirable texts by Wilks (*The Properties of Liquid and Solid Helium*; Clarendon: Oxford, 1967) and by Keller (*Helium-3 and Helium-4*; Plenum: New York, 1969) are thus almost certainly the last of their kind. In presenting the fruits of more recent researches, Bennemann and Ketterson have very sensibly chosen to solicit individual chapters from a selection of experts. Although inevitably lacking the coherence, internal self-consistency and the helpful cross-referencing associated with a single-author monograph, the result is a collection of weighty, authoritative and, on the whole, very interesting review articles.

This second volume is strongly biased towards helium-3 which is, of course, entirely appropriate given the enormous progress made towards elucidating the properties of its super-

fluid phases following their discovery in 1972. The first three chapters are tough going, relatively speaking, being mostly devoted to a formal development of the theory: Baym and Pethick deal with the theory of normal (non-superfluid) liquid ^3He and with ^3He - ^4He solutions; Anderson and Brinkman cover anisotropic superfluidity in liquid ^3He . Most graduate students would be well advised to start instead on chapter 4, by Lee and Richardson. Running to more than 200 pages their contribution is virtually a book in its own right. They explain and discuss in considerable detail almost all the experimental work carried out on superfluid ^3He up to a cut-off date in mid 1976, conveying in an admirable way the relationship between the experiments

and the growing theoretical structure.

The remaining chapters are devoted to articles on three very disparate topics: by Dash and Schick on two-dimensional helium, concentrating, in particular, on the recent fascinating investigations of helium adsorbed on grafoil substrates; by Brewer on multi-layer helium films in restricted geometries; and by Price on neutron scattering from helium. Each of these is excellent in its own way and, like the articles on ^3He , they should be a real help both to people entering, as well as to those already working in, the field in question.

The quality of production is high, as indeed it should be, considering the price. Very few private individuals will be able to afford personal copies; but the book provides a substantial and probably lasting contribution to the literature on condensed state helium and, as such, it will obviously be an essential purchase for libraries.

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Bile salts

The Biological Importance of Bile Salts. By G. A. D. Haslewood. Pp.206. (North-Holland: Amsterdam, New York and Oxford, 1978.) \$55.75; Dfl.125.

THE interest in bile salts of the author of this useful publication is of very long standing. However, the author points out in the preface that this volume does not represent a second edition of his earlier publication entitled *Bile Salts* (1967). As remarked later in the present book, its purpose is to put the reader into the biological picture (physiological, biochemical, taxonomic and medical) regarding bile salts.

How can such a slim volume, with only some 200 pages, expect to achieve this aim? Professor Haslewood has succeeded by being selective. The book does not, for example, deal in any detail with the chemistry of bile salts, and the references (up to 1978) are described as a personal choice selected partly for their general interest. The author considers that, in these times of computer storage and retrieval, completeness seems an unnecessary aim and may make reading difficult and dull. Several chapters therefore commence by providing appropriate documentation to comprehensive earlier sources for background information.

The monograph has been produced to a high standard, with few printer's errors. It has five main chapters, of which the longest reflects the author's interest in the distribution of bile salts in the animal kingdom—for example, "Thus, crocodilian bile is quite primitive and generalised: a halt on the main route of bile salt evolution". The other chapters deal with the nature and function of bile, chemistry and methods of separation and analysis of bile salts, their biosynthesis and artefacts of the enterohepatic circulation, and their medical importance.

An Appendix contains 16 pages of tabulated data consistent with the intention that, throughout their studies, the author and his colleagues have tried to publish records of all bile salts discovered. The possible medical relevance of such compilations is shown by the observation (p189) that the tree "shrew" has advantages as a laboratory animal since this primate has bile much more like man than other small laboratory species, and can easily be induced to form cholesterol gallstones.

This monograph will thus be useful to many workers concerned with a number of different aspects of bile salts in biology and medicine.

J. A. Lucy

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Earth sciences

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BOGLI, Alfred. Karsthydrographic und Physische Speläologie. Pp.xii + 292. ISBN-3-540-09015-0. (Berlin and New York: Springer-Verlag, 1978.) DM 58; \$31.90.

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HUSAR, R. B., LODGE, Jr., J. P., and MOORE, D. J. (edited by). Sulfur in the Sulfur in the Atmosphere. (Proceedings of the International Symposium held in Durhrovnik, Yugoslavia, 7-14 September 1977.) Pp.816. ISBN-0-08-022932-8. (Oxford and New York: Pergamon Press, 1978.) £15.

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ROBERTS, P. H., and SOWARD, A. M. (edited by). Rotating Fluids in Geophysics. Pp.xvii + 551. ISBN-0-12-589650-6. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) £17.50; \$36.25.

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Biology

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ANDERSON, John. Sulphur in Biology. (The Institute of Biology's Studies in Biology, No. 101.) Pp.59. ISBN-0-7131-2711-2. (London: Edward Arnold (Publishers), Ltd., 1978.) £1.60 net.

ANGELL, Tony. Ravens, Crows, Magpies and Jays. Pp.112. ISBN-0-295-9589-9. (Seattle and London: University of Washington Press, 1978.) £10.50.

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BARASH, David P. Sociobiology and Behavior. Pp.xv + 378. ISBN-0-435-62046-0. (London: Heinemann Educational Books, Ltd., 1978.) £3.95 net.

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BLACKBURN, S. (edited by). Amino Acid Determination: Methods and Techniques. Second edition, revised and expanded. Pp.x + 365. ISBN-0-8247-6349-1. (New York and Basel: Marcel Dekker, Inc., 1978.) Sw.fr.82.

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CLOUDSLEY-THOMPSON, J. L. Why the Dinosaurs Became Extinct (Patterns of Progress: Zoology Series.) Pp.85. ISBN-0-9004095-29-0. (Shildon, Co. Durham: Meadowfield Press, Ltd., 1978.) UK £2.80; Overseas \$8.40.

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DAVIES, Donald G., and BARNES, Charles D. (edited by). Regulation of Ventilation and Gas Exchange. (Research Topics in Physiology.) Pp.xii + 308. ISBN-0-12-204650-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) £22; £14.30.

ELLENBERG, Heinz. Vegetation Mitteleuropas mit den Alpen in Ökologischer Sicht. Zweite, völlig neu bearbeitete Auflage, 499 Abbildungen und 130 Tabellen. Pp.982. ISBN-3-8001-3418-7. (Stuttgart: Verlag Eugen Ulmer, 1978.) DM 120.

FABER, Donald S., and KORN, Henri (edited by). Neurobiology of the Mauthner Cell. Pp.xii + 290. ISBN-0-89004-233-0. (New York: Raven Press, 1978.) \$32.50.

FALKNER, Frank, and TANNER, J. M. (edited by). Human Growth. Vol.2: Postnatal Growth. Pp.xviii + 634. ISBN-0-306-34462-9 (v.2). (New York and London: Plenum Press, 1978.) £22.05.

FREIFELDER, David. Molecular Biology and Biochemistry: Problems and Applications. (A Series of Books in Biology.) Pp.xv + 308. ISBN-0-7167-0068-9. (San Francisco and Reading: W. H. Freeman and Company, 1978.) £4.10.

GARLAND, P. B., and HALES, C. N. (organized and edited by). Substrate Mobilization and Energy Provision in Man. (Biochemical Society Symposium No. 43, held at University College, Cardiff, July 1977.) Pp.x + 228. ISBN-0-904498-07-7. (London: The Biochemical Society, 1978.) £15; \$32.

GILLHAM, Nicholas W. Organelle Heredity. Pp.xv + 602. ISBN-0-89004-102-4. (New York: Raven Press, 1978.) \$64.35.

GUINCHET, Marcel, et de VILMORIN, Roger. Flore de France, Fascicule 3. Pp.819-1199. ISBN-2-222-02171-5. (Paris: Éditions du Centre National de la Recherche Scientifique, 1978.) F.80.

HALL, Brian K. Developmental and Cellular Skeletal Biology. Pp.x + 304. ISBN-0-12-318950-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) £21; £13.65.

HARRISON, R. J., and RIDGWAY, Sam H. Deep Diving in Mammals. (Patterns of Progress: Zoology Series.) Pp.51. ISBN-0-900-54189-X. (Shildon, Co. Durham: Meadowfield Press, Ltd., 1976.) UK £2.80; Overseas \$8.40.

HAZELBAUER, G. L. (edited by). Taxid and Behavior: Elementary Sensory Systems in Biology. (Receptor and Recognition, Series B, Volume 5.) Pp.x + 341. ISBN-0-470-26483-7. (London: Chapman and Hall, 1978. Distributed in the USA by Halsted Press, a Division of John Wiley and Sons, Inc., New York.) £18.

announcements

Meetings

9–10 April, **Enzyme Inhibitors as Drugs**, London (Mrs J. Kruger, Dept of Pharmacology, School of Pharmacy, Brunswick Square, London WC1).

11 April, **Computers in Drug Research and Development**, London (Society for Drug Research, c/o Institute of Biology, 41 Queen's Gate, London SW7, UK).

17–18 April, **1st European Conference on the Protection of Farm Animals**, Amsterdam (Barban Public Relations Ltd, 43 Great Marlborough Street, London, W1, UK).

18–19 April, **The Terrestrial Environment and the Origin of Land Vertebrates**, Newcastle upon Tyne (Dr A. L. Panchen, Dept of Zoology, The University, Newcastle upon Tyne, UK).

23–24 April, **Stereodynamics of Molecular Systems**, New York (Dr R. H. Sarma, State University of New York at Albany, 1400 Washington Avenue, Albany, New York 1222).

29 April–2 May, **Ceramic Quality, Durability and Reliability**, Cincinnati (American Ceramic Society, 65 Ceramic Drive, Columbus, Ohio 43214).

10 May, **Safety in Research Laboratories**, London (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

22–25 May, **13th International Leuko-**

cyte Culture Conference, Ottawa (K. Charbonneau, c/o National Research Council of Canada, Ottawa, Canada KIA OR6).

10–30 June, **The Transformed Cell**, New York (Cold Spring Harbor Laboratory, New York 11724).

10–30 June, **Advanced Bacterial Genetics**, New York (Cold Spring Harbor Laboratory, New York 11724).

21–22 June, **Lysosomes in Endocrine Secretion**, Marseille (Prof. C. Simon, Laboratoire d'Endocrinologie Cellulaire, Université de Provence, Centre St-Charles, 3 Place Victor Hugo, 13331 Marseille Cedex 3, France).

25–27 June, **Amino Acid Analysis in Clinical Chemistry and Medical Research**, Edinburgh (Dr J. M. Rattenbury, Dept of Paediatric Biochemistry, Royal Hospital for Sick Children, Edinburgh, UK).

27–29 June, **2nd International Conference on Lubrication Challenges in Metal-Working and Processing**, Illinois (IIT Research Institute, 10 West 35 Street, Chicago, Illinois 60616).

27–29 June, **Implantable Transducers and Systems: Packaging Methods and Testing Criteria**, Stanford (Prof. J. B. Angell, Electrical Engineering Dept, Stanford University, California 94305).

3–23 July, **RNA-TV**, New York (Cold Spring Harbor Laboratory, New York 11724).

3–23 July, **Molecular Biology and Developmental Genetics of Drosophila**, New York (Cold Spring Harbor Laboratory, New York 11724).

25 June–7 July, **Medical Physics**, Varenna (Prof. M. Della Corte, Istituto di Fisica Medica e Medicina Nucleare, Viale Morgagni, 85, 50134 Firenze, Italy).

9–21 July, **Nuclear Structure and Heavy Ion Collisions**, Varenna (Prof. R. A. Ricci, Istituto di Fisica Galileo Galilei, Università di Padova, Via Marzola, 8, 35100 Padova, Italy).

23 July–4 August, **Physics of the Earth's Interior**, Varenna (Prof. E. Boschi, Istituto di GEOFISICA dell'Università, Via Irnerio, 46, 40126 Bologna, Italy).

25 July–14 August, **Yeast Genetics**, New York (Cold Spring Harbor Laboratory, New York 11724).

30 July–12 August, **Immunogenetics**, New York (Cold Spring Harbor Laboratory, New York 11724).

13–16 August, **Impact of Intensive Harvesting on Forest Nutrient Recycling**, Syracuse (Dr A. L. Leaf, SUNY College of Environmental Science and Forestry, Syracuse, New York 13210).

24–30 August, **The Ammonoidea**, York (Dr J. R. Senior, Dept of Extra-Mural Studies, 32 Old Elvet, Durham, UK).

Gordon Research Conferences; Frontier of Science; 1979 Schedule—New Hampshire

	Colby-Sawyer College New London, NH	New Hampton School New Hampton, NH	Kimball Union Academy Meriden, N.H.	Tilton School Tilton, NH	Proctor Academy Andover, NH	Holderness School Plymouth, NH	Brewster Academy Wolfeboro, NH	Plymouth State College Plymouth, NH
11–15 June	*Elastin	Nucleic Acids	Cyclic Nucleotides	Theoretical Biology and Biomathematics	Free Radical Reactions	Physical Organic Chemistry (Hydrocarbon Chemistry)	Space Plasma Physics	*Red Cells
18–22 June	Nuclear Chemistry	Environmental Sciences: Air Proteins	Lipid Metabolism	Animal Cells and Viruses	Plant Cell and Tissue Culture	Mammary Gland Biology	Magnetic Resonance Atomic Physics	Calcium Phosphates
25–29 June	Catalysis		Atherosclerosis	Carbohydrates, Chemistry of Polymer Colloids	Polyamines	Biological Regulatory Mechanisms	Nonlinear Optics	Molecular Pharmacology
2–6 July	Fiber Science	Bacterial Cell Surfaces	Interfaces, Chemistry at Bones and Teeth, Chemistry, Physiology and Structure of Enzymes, Coenzymes and Metabolic Pathways	Nuclear Structure Physics	Cell Adhesion and Movement	*Analytical Pyrolysis		Fuel Science
9–13 July	Polymers	Heterocyclic Compounds, Chemistry of	Point and Line Defects in Semiconductors		Physical Metallurgy	Solids, Chemistry and Physics of	Molecular Pathology	Quantum Solids and Fluids, Dynamics of
16–20 July	Elastomers	Organic Reactions and Processes	Safety Evaluations	Biomaterials, Science and Technology of *Food Microbiology	Energy Coupling Mechanisms	Chemotherapy of Experimental and Clinical Cancer	Macromolecules and Behavior	Quantitative Structure Analysis
23–27 July	Corrosion	Natural Products			Organic Photochemistry	Drug Metabolism	Microbiological Degradation	Tumor Immunology
30–3 July	Food and Nutrition	Statistics in Chemistry and Chemical Engineering		Muscle Ionic Channels in, and Other Excitable Membranes	Developmental Biology	Fertilization and the Activation of Development	Ceramics, Solid State Studies in	*Ion Containing Polymers
6–10 August	Medicinal Chemistry	Inorganic Chemistry	Glycoproteins and Glycolipids	*Epithelial Differentiation and Keratinization	Coatings and Films, Chemistry and Physics of Catecholamines	Postharvest Physiology	Micellar and Macromolecular Catalysis	Magnesium in Biochemical Processes
13–17 August	Separation and Purification	Analytical Chemistry	Fluids in Permeable Media	Laser Interaction with Matter		Liquids, Chemistry and Physics of Inorganic Geochemistry	Few Body Problems in Chemistry and Physics	*Dynamics of Gas-Surface Interactions
20–24 August	Cancer	Adhesion, Science of	Ion Exchange	Transport Phenomena in Lipid Bilayer and Biological Membranes	Elementary Particle Interactions		Molten Salts and Metals	*Remote Sensing of the Earth's Surface from Space

Contact: Dr. A. M. Cruickshank, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881.

Reports and Publications

UK and Ireland — February

- Countryside Commission. New Long Distance Path Leaflets. Offa's Dyke Path. The Cleveland Way. Pembrokeshire Coast Path. The Penine Way. North Downs Way. South Downs Way. South Devon Coast Path. The Ridgeway Path. Cornwall Coast Path. Dorset Coast Path. Somerset and North Devon Coast Path. (Cheltenham, Gloucestershire: The Countryside Commission, John Dower House, Crescent Place, 1979.) *gratis*. [12]
- Nutrition in Eye Health and Disease. By Stanley C. Evans. Pp. 38. (London: Roberts Publications, 225 Putney Bridge Road, SW15, 1978.) £1.50. [12]
- The Association of Commonwealth Universities. Annual Report of the Council 1977-78. Pp. 50. (London: The Association of Commonwealth Universities, 36 Gordon Square, WC1, 1979.) [12]
- United Kingdom of Great Britain and Northern Ireland. Report on the Implementation of the Convention on International Trade in Endangered Species of Wild Fauna and Flora in the United Kingdom, 1 January-31 December 1977. Pp. 74. (London: Department of the Environment, 2 Marsham Street, SW1, 1979. Available from Department of the Environment, Wildlife Conservation Licensing Section, Tollgate House, Houlton Street, Bristol.) [12]
- Ministry of Overseas Development. Report on Research and Development. 1978. Pp. 76. (London: HMSO, 1978.) £2 net. [22]
- Health and Safety Executive. Health and Safety Statistics, 1976. Pp. vi+65. (London: HMSO, 1979.) £1.75 net. [22]
- Tropical Products Institute. Grain Processing Losses Bibliography: Covering Threshing, Shelling, Hulling, Milling, Grinding etc. and Excluding Harvesting and Storage. By Ruth Kasasian and D. A. V. Dendy. Pp. iv+47. (G117). (London: Tropical Products Institute, 56/62 Gray's Inn Road, WC1, 1979.) £1.35. [22]
- The Glasshouse Crops Research Institute. Annual Report, 1977. Pp. 226. (Rustington, Littlehampton, West Sussex: The Glasshouse Crops Research Institute, 1978.) [52]
- The Universities Central Council on Admissions. Sixteenth Report, 1978-77. Pp. 28. (Cheltenham, Glos.: The Universities Central Council on Admissions, 1979.) 65p. [52]
- The Hannah Research Institute. (University of Glasgow). Report 1978. Pp. 126. (Ayr, Scotland: The Hannah Research Institute, 1979.) £2.50. [72]
- Health and Safety Executive. Packaging and Labelling of Dangerous Substances: Regulations and Guidance Notes. Pp. iii+23. (London: HMSO, 1978.) 60p plus postage. [82]
- Health and Safety Executive. Safety in the Use of Timber Pallets. (Guidance Note PM 15.) Pp. 8. (London: HMSO, 1979.) 30p plus postage. [82]
- Department of the Environment: Central Unit on Environmental Pollution. Pollution Control in Great Britain: How It Works. (Pollution Paper No. 91) Pp. vi+107. (London: HMSO, 1978.) £2.25 net. [92]
- The Supply of Potential Technicians, Craftsmen and Operators from the Education System to the Engineering Industry. By T. Fidgett. Pp. 84. (RP/3/78). (Watford: Engineering Industry Training Board, 54 Clarendon Road, 1978.) [92]
- An Introductory Guide to Information Sources in Physics. By L. R. A. Melton. Pp. 44. (Bristol and London: The Institute of Physics, 1978.) £1.25. [122]
- Advisory Council for Applied Research and Development. Industrial Innovation. Pp. 43. (London: HMSO, 1978.) £1 net. [121]
- Health and Safety Commission. Proposal to Amend the Agriculture (Tractor Cabs) Regulations, 1974, to Align with EEC Special Directives on Noise Requirement and Roll Over Protection Structures (Safety Cabs). (Consultative Document). Pp. 7. (London: HMSO, 1979.) 20p net. [122]
- Report of The Rugby School Natural History Society for the year 1977-78. (One Hundred and Eleventh Issue). Pp. 44. (Rugby: The Rugby School Natural History Society, 1979.) [122]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 290, No. 1376: Pathways of Pollutants in the Atmosphere. A Discussion organized by T. M. Sugden, F.R.S., for the Royal Society's Study Group on Pollution in the Atmosphere. Pp. 467-637+ 1 plate. (London: The Royal Society, 1979.) UK £11.20; Overseas £11.70. [122]
- Medical Teacher. Vol. 1, No. 1, January/February 1979. Published bi-monthly. Pp. 1-56. Subscription: £13; \$32. (London: Update Publications, Ltd., Alfred Place, WC1; Fort Lee, New Jersey: Update Publishing International, Inc., 2337 Lemoine Avenue, 1979.) [132]
- Life and Death Before Birth: a Study of the Human Problems Arising from the Use of Antenatal Diagnostic Techniques. Pp. 64. (London: Council for Science and Society, 3/4 St. Andrews Hill, 1979.) £1.50. [132]
- Natural Environment Research Council: Institute of Terrestrial Ecology. Biological Records Centre. By John Heath and Franklin Perring. Pp. 20. (Huntingdon: Institute of Terrestrial Ecology, Monks Wood Experimental Station, 1978.) 80p net. [162]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 285, No. 1005: The Primitive Cynodont *Procyonotuchus*: Functional Anatomy of the Skull and Relationships. By T. S. Kemp. Pp. 73-122+plates 1-3. (London: The Royal Society, 1979.) UK £4.28; Overseas £4.50. [192]
- Geological Survey of Northern Ireland. Department of Commerce. Geology of the Causeway Coast. By

- H. E. Wilson and P. I. Manning. (Memoir for One-Inch Geological Sheet, 7.) Vol. 1: Pp. X+1-72+plates 1-23. £2.95 net. Vol. 2: Pp. vii+73-172+plates. 24-37. £13 net. (Belfast: HMSO, 1978.) [192]
- National Radiological Protection Board. NRPB-R73: The Influence of the Physico-Chemical Form of the Aerosol on the Radiological Consequences of Notional Accidental Releases of Radioactivity from a Fast Breeder Reactor. By G. N. Kelly, J. A. Jones and J. R. Simmonds. Pp. iv+145. (Harwell, Didcot: National Radiological Protection Board, 1979. Obtainable from HMSO.) £3. [192]
- The Parliamentary and Scientific Committee. Annual Report 1978. Pp. 24. (London: Secretary, The Parliamentary and Scientific Committee, 30 Farringdon Street, EC4, 1979.) £1. [202]
- Countryside Commission. Countryside Bill 1978: Comments by the Countryside Commission. Pp. 12. (Cheltenham, Glos.: Countryside Commission, John Dower House, Crescent Place, 1979.) *gratis*. [202]
- Ministry of Agriculture, Fisheries and Food. Report of the Director of Fisheries Research, 1974-77. Pp. 80. (Lowestoft, Suffolk: Ministry of Agriculture, Fisheries and Food, Directorate of Fisheries Research, 1978.) [202]
- Third Report of the Advisory Board for the Research Councils, 1976-1978. Pp. vii+27. (Cmd. 7467). (London: HMSO, 1979.) 70p net. [212]
- Professional Ideals and Planning Practice. By Patsy Healey and Jacky Underwood. Pp. 73-127. (*Progress in Planning*, Vol. 9, Part 2.) £4. An Analysis of New Industry Location—The Irish Case. By P. N. O'Farrell. Pp. 139-229. (*Progress in Planning*, Vol. 9, Part 3.) £4. (Oxford and New York: Pergamon Press, 1978.) [212]
- Scarce Resources in Health Care. Pp. 24. (London: Office of Health Economics, 162 Regent Street, W1, 1979.) 35p. [222]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 290, No. 1377: Nonlinear Wave Packets in the Kelvin-Helmholtz Instability. By M. A. Weissman. Pp. 639-685. (London: The Royal Society, 1979.) UK £2.38; Overseas £2.58. [222]
- Compare Your Home Heating Costs. (An Independent Guide to the Costs of Different Fuels and Heating Methods and How to Reduce Them.) Pp. 17. (London: Library of Energy, Thames House South, Millbank, SW1, 1979.) *gratis*. [222]
- Waste Management Advisory Council: Waste as Fuel Working Party. Energy from Waste. Pp. 47. (London: HMSO, 1979.) £1.50 net. [232]
- Enzyme and Microbial Technology. Vol. 1, No. 1, January 1979. Managing Editor: Christopher J. Rawlins. Assistant Editor: Monique Heald. Published quarterly. Pp. 1-72. Annual subscription, including postage by surface mail: £40 UK and overseas. Single copies: £10. (Haywards Heath, Sussex: IPC (Sales and Distribution), Ltd., Oakfield House, Perry Mount Road, 1979.) [262]
- Ministry of Overseas Development. Overseas Development Paper No. 14: Development Education. (Report and Recommendations by Working Party of the Advisory Committee on Development Education.) Pp. vii+52. (London: HMSO, 1979.) [262]
- Survey of Attitudes Towards Overseas Development. Submitted to The Central Office of Information, London, SE1. Submitted by The Schlackman Research Organization, Ltd., London, NW8. Pp. 158. (London: HMSO 1978.) £7 net. [262]
- Amoco Cadiz Oil Spill. Guest Editor: Molly F. Spooner. (*Marine Pollution Bulletin*, Vol. 9, No. 11, November 1978.) Pp. 281-311. (Oxford and New York: Pergamon Press, 1978.) £3; \$6. [272]
- Department of Industry: Committee on Corrosion. Corrosion Control in Engineering Design. Pp. 34. (London: HMSO, 1978.) £1.85 net. [282]

Other countries — January

- Fisheries and Environment Canada. Fisheries Marine Service. Technical Report No. 822: Bay of Fundy Environmental and Tidal Power Bibliography. By Catherine M. Moysse. Pp. v+36. (St. Andrews, N.B.: Biological Station, 1978.) [151]
- Landeshauptstadt Stuttgart. Beiträge zur Stadtentwicklung 15: Daten und Aussagen zum Stadtklima von Stuttgart auf der Grundlage der Infrarot-Thermographie—Eine Wissenschaftliche Abhandlung zum Planungsfaktor Stadt- und Landschaftsklima. Von Dr. Franz Robel, Ulrich Hoffman and Anselm Rieker. Pp. 260. (Stuttgart: Chemisches Untersuchungsamt der Landeshauptstadt Stuttgart, Klimatologische Abteilung, 1978.) [171]
- Democratic Republic of the Sudan. Ministry of Agriculture: Agricultural Research Corporation. Annual Report of Kenana Research Station, 1973/1974. Pp. 135. Annual Report of the Hudelba Research Station, 1966/1967. Pp. 84. (Wad Medani, Republic of the Sudan: Agricultural Research Corporation, Gezira Research Station, 1978.) [191]
- Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 148, No. 3: The American Orb-Weaver Genera *Cyclosa*, *Metazygia* and *Eustala* North of Mexico (Araneae, Araneidae). By Herbert W. Levi. Pp. 61-127. Vol. 148, No. 8: Natural History of *Cerion* VIII: Little Bahama Bank—a Revision Based on Genetics, Morphometrics, and Geographic Distribution. By Stephen Jay Gould and David S. Woodruff. Pp. 371-415. \$3.75. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1977 and 1978.) [231]
- CERN — European Organization for Nuclear Research. CERN 78-13: Proceedings of the 1978 CERN School of Computing, Jadwisin, Poland, 28 May-10 June 1978. Pp. ix+236. List of Scientific Publications 1976. Pp. 30. List of Scientific Publications 1977. Pp. 29. (Geneva: CERN, 1978.) [231]

- Sur la Distribution Géographique des Points Chauds de la Terre. Par Georges Dubourdieu. Pp. 45. (Meudon, France: Collège de France, Laboratoire de Géologie Avenue Marcelin Berthelot, 1979.) [231]
- World Health Organization. A Coded Compendium of the International Histological Classification of Tumours. Edited by L. H. Sobin, Louis B. Thomas, Constance Percy and Donald E. Henson. Pp. 116. Sw.fr. 25. International Classification of Procedures in Medicine, Vol. 2. 3. Radiology and Certain Other Applications of Physics in Medicine. 6, 7. Drugs, Medicaments, and Biological Agents, Various paginations. Sw.fr. 15. (Geneva: WHO; London: HMSO, 1978.) [291]
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